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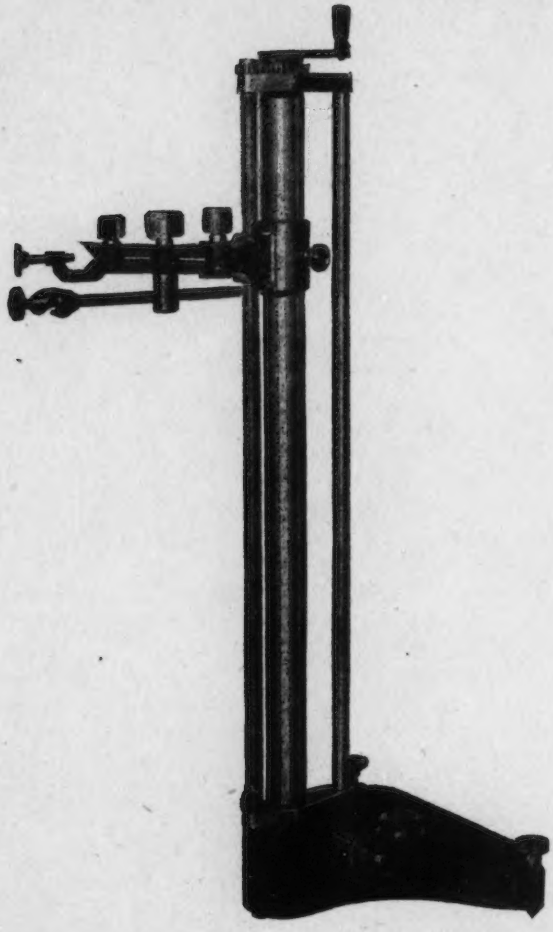

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THE EFFECTS OF CARBON ARC RADIATION ON THE BLOOD OF DOGS[1]

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Changes in the physical characters of the blood of normal dogs on exposure to measured amounts of C arc radiation were reported in earlier studies (Miles and Laurens, 1926). With moderate dosage there was an initial increase in reds followed by a decrease; with strong dosage, an immediate decrease; and with both dosages, a gradual increase in the post-irradiation period to a new level 1,500,000 above normal. These results were taken to indicate a stimulation of the hematopoietic system, the effect being at first obscured by increased blood volume, an opinion strengthened by the finding of decreased total solids and Hb percentages, both of which reached normal shortly after the irradiation period.

The experiments here reported are in continuation of this work on normal adult animals, preliminary to a study of the effect of radiation on experimental anemia. Eight white short-haired dogs on a standard maintenance diet (Cowgill, 1923) were used. Blood was obtained from a freely flowing ear puncture, the first few drops being discarded. Simultaneous samples from the femoral vein and artery, or the toe-pad, gave similar counts. To minimize the effect of diurnal variations, determinations were always made at the same hours of the day on each animal, one in the morning immediately preceding the irradiation, a second immediately after, and a third in the afternoon about 5 hours after the end of the irradiation. Where massive exposures were used, the irradiation was interrupted for a short time and an additional count made during the irradiation period. The same procedure (minus the irradiation) was

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followed for at least a week at the beginning of each experiment to determine the normal levels. The animals were irradiated abdominally with the Pan-Ray-Arc, the eyes being protected. During the observations here reported this lamp, when operated at 25–28 A., and 55–60 V., emitted 0.8 g. cal. per sq. cm. per min. of energy incident at 1 M. The approximate distribution was: 12 per cent in the ultra violet; 35 per cent in the visible; and 53 per cent in the infra red.

Red and white cell counts were made in the usual manner. The same pipettes were used throughout and duplicate counts made by the same person. Platelets were counted by Pratt's method as modified by Liles (1926), hemoglobin was determined with the Newcomer hemoglobinometer, and cell and platelet volume by the methods of Van Allen (1925, 1926). In six of the experiments changes in plasma volume were determined by the dye method of Hooper, Belt, Smith and Whipple (1920) and specific gravity according to Barbour and Hamilton (1926). In the last three experiments repeated plasma volume determinations were made immediately before and after the exposure as well as several days later by the method of Smith (1920). In the other experiments, values obtained immediately after irradiation were compared with determinations obtained in the normal pre-irradiation period.

The recommendations of Osgood (1926, 1927) have been followed in the calculations of the color, volume and saturation indices. By this method all values are referred to an arbitrary standard of 5 million red cells per cmm. The color index is obtained by dividing Hb percentage by red cell percentage. For this calculation, the normal Hb coefficient (grams Hb per 100 cc. blood per 5 million reds) determined for each dog during the pre-irradiation period is considered as 100 per cent. The volume index is here expressed as the ratio between the size of the red cells during the irradiation and post-irradiation periods and the average size in the pre-irradiation period. It is obtained by dividing the volume of cells (hematocrit) expressed as percentage of the normal volume (100 per cent being considered as the average volume of cells in 100 cc. of blood calculated to a red count of 5 millions, the volume coefficient) by the percentage of the reds (5 million being 100 per cent). The saturation index expresses the ratio between the concentration of Hb per unit volume of cells and the average concentration of Hb per unit volume of cells in the pre-irradiation period. It is obtained by dividing the color index by the volume index.

Experiment 1. After a 10 day preliminary period, determinations were begun on November 20. With the exception of the whites, which fluctuate somewhat, the diurnal variations are slight. Beginning on December 1, dog 1 was irradiated abdominally 13 times daily as follows: $\frac{3}{4}$ hour at 1 M. (36 g. cal. per sq. cm.) for 3 days; $\frac{3}{4}$ hour at 90 cm. (45 g. cal. per sq. cm.) for 5 days; $\frac{3}{4}$ hour at 80 cm. (56 g. cal. per sq. cm.) for a single exposure,

followed by 4 massive doses of 1 hour at 60 cm. (133 g. cal. per sq. cm.). The first irradiation results in a marked increase in the blood volume as evidenced by a uniform dilution of all the constituents immediately after irradiation (fig. 1 *a*). This decrease is of the order of 34 per cent in the red cells, 33 per cent in the platelet number and volume, 27 per cent in the Hb, 28 per cent in the hematocrit, and 12 per cent in the whites. The proportionately smaller decrease in the latter is interpreted as a probable increase in number partly masked by the dilution. Although the change in the color index is slight (1.0 to 0.90) the considerable increase in the volume index (1.0 to 1.26) results in a low saturation index (0.79), that is,

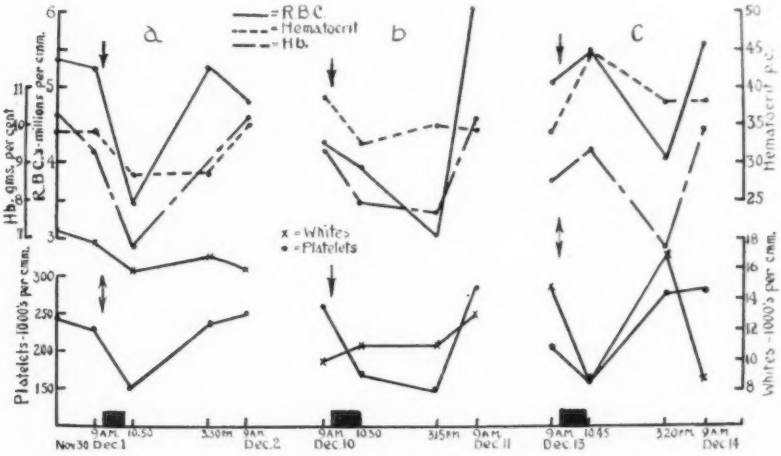

Fig. 1. Typical effects following irradiation. Arrows and heavy blocks show beginning and approximate duration of exposures. *a*. First irradiation ¾ hour at 1 M (36 g. cal. per sq. cm.). The first points indicate average normal pre-irradiation values. *b*. Tenth daily irradiation—1 hour at 60 cm. (133 g. cal. per sq. cm.). *c*. Thirteenth daily exposure, dose same as in *b*.

there is a decreased concentration of Hb per unit volume as compared with the normal. The values return to approximately pre-irradiation levels within 5 hours after the exposure, with the exception of the hematocrit, which does not return to normal until the next morning.

This diffusion of water from the tissues to the blood accompanies each subsequent irradiation as evidenced by the remarkably uniform decrease (about 35 per cent) in the platelet number and volume during the exposure, with recovery to normal or above by the afternoon of the same day. The responses of the other constituents on subsequent irradiation are more varied. Red cell number and volume and Hb show decreases of 6 to 36

per cent during the first nine, and again during the eleventh, exposures, with recovery each time to normal or above in the afternoon of the same day or the morning of the next. The first massive (tenth) irradiation is followed by an 8 per cent decrease in reds which is intensified to 25 per cent in the afternoon, followed by a marked (25 per cent above normal) increase the next morning (fig. 1 *b*). The decrease here observed is due to a persistent dilution resulting from the extreme dose, for all values remain at low levels in the afternoon. The last two irradiations result in an actual increase in reds to 7 per cent above the pre-irradiation value and corresponding increases in hematocrit and Hb observable during the exposure, followed by a decrease of about 15 per cent in the afternoon and recovery to normal or higher levels on the next day (fig. 1 *c*). That the increase during irradiation is not due to blood concentration is shown by the usual drop in the platelets as well as a decrease in the whites during the irradiation with recovery in the afternoon. The subsequent decrease may be due to increased fragility of the newer cells. The whites show a consistently decreasing level throughout the experiment, the value on the day after the last exposure being only 50 per cent of the pre-irradiation value. In the majority of cases they decrease slightly during the exposure and rise again in the afternoon. This is especially marked for the last massive exposure, there being a 100 per cent increase in the afternoon, followed, however, by a return to the low level the next morning (fig. 1 *c*). The increase in rectal temperature at this time (1.5°) would indicate that the leucocytosis is due to a possible inflammatory reaction brought about by the intense radiation. The differential changes during the irradiation period are inconsistent. The first two exposures are followed by a relative lymphocytosis which, however, gives way to a lymphopenia persisting throughout the irradiation period. Neutrophiles and eosinophiles usually increase during and after irradiation but the effect is not consistent.

The last exposure was given December 13, and the animal followed for ten days after. The red cell volume and number are high on the morning after the last irradiation and increase still more to a maximum of 22 per cent and 25 per cent respectively above pre-irradiation values on December 15, followed by a gradual decrease to normal 7 days after. While the changes in Hb during the first part of the irradiation period closely parallel those in the red cells, they fail to do so in the post-irradiation period, remaining at a level about 89 per cent normal. The increases in the cell volume, on the other hand, are greater than those in the number, especially on the morning after the exposures and during the last four massive irradiations, when the cell volume remains high during the irradiations. This increased size accompanied by a low Hb content (average saturation index 0.84) is a reaction resembling that frequently seen in regeneration from hemorrhagic anemia where the production of Hb does

not keep pace with the formation of stroma (Whipple and Robscheit-Robbins, 1925). Except for the consistent decrease with each irradiation, platelets show little change until after the eighth exposure, which is followed by a high count in the afternoon, reaching a maximum of 17 per cent above normal in the afternoon following the ninth exposure. The decrease during irradiation, however, is still of the same magnitude (35 per cent). The high count persists for 1 day after irradiation and quickly drops to normal. The changes in platelet volume in general parallel those in the count. The white cell level, as previously discussed, decreases gradually throughout and is at a level about 40 per cent below normal during the post-irradiation period. The differential in this period shows a definite lymphocytosis, an increase in eosinophiles, and a drop in neutrophiles of 9 per cent lasting for about a week in contrast to the lymphopenia and

TABLE 1

Average values for 1 week before and after irradiation

	REDS	Hb	COLOR INDEX	HEMATOCRIT	VOLUME INDEX	SATURATION INDEX	PLATELETS	TOTAL WHITES	LYMPHOCYTES	NEUTROPHILES	EOSINOPHILES
	millions	*grams per cent*		*per cent*			*in 1000's*		*per cent*	*per cent*	*per cent*
Pre-irradiation.....	4.86	10.04	1.00	31.8	1.00	1.00	244	16,440	23	70	7
Post-irradiation.....	5.79	8.98	0.79	36.5	0.96	0.84	254	9,770	25	64	11
Difference..	+0.93	−1.06	−0.21	+4.7	−0.04	−0.16	+10	−6,670	+2	−6	+ 4
Per cent change...	+19.00	−11.00	−21.00	+15.0	−4.00	−16.00	+4	−41	+8	−9	+57

increased neutrophile count during the latter part of the irradiation period. The average values of the constituents for the pre- and post-irradiation periods are given in table 1.

Experiments 2, 3, 4 and 5. The efficacy of the massive doses employed in the last part of the previous experiment in effecting marked responses of the hematopoietic system suggested the possibility of maintaining or augmenting these changes by similar doses repeated at intervals. Dogs 2 and 3 were accordingly exposed to three irradiations of one-half hour at 60 cm. (67 g. cal. per sq. cm.) at intervals of 2 and 3 days respectively, followed in 6 days by an exposure of three-quarters of an hour at the same distance (100 g. cal. per sq. cm.). In experiments 4 and 5, in order to reduce the apparent heat effects, the animals (dogs 4 and 5) were irradiated with the same total energy (67 g. cal. per sq. cm.) at the same intervals but at a greater distance away from the lamp (53 min. at 80 cm.).

Allowing for individual variations and duration of exposure, the results of these experiments, as well as those of the first, are in close agreement. The first irradiation in each animal is accompanied by comparable decreases in reds, Hb and hematocrit of from 7 to 37 per cent, reaching a maximum either immediately or within 5 hours after the exposure. In experiments 2, 3 and 4 the decreases are of much smaller magnitude (5 to 17 per cent) than observed following the first irradiation in experiment 1. On the other hand, platelets and whites decrease 37 and 34 per cent respectively in dog 2; 20 and 23 per cent in dog 3; and 12 and 21 per cent in dog 4, indicating that the dilution here is of similar magnitude to that of experiment 1, and is masking an actual increase in the other constituents. Unless we admit of increased formation of red cells accompanying the dilution, we must assume in these cases a greater destruction of platelets and whites than of reds and hemoglobin, a supposition which is hardly in accord with the evidence at hand, especially since the former constituents are back to normal at most within 8 hours. The changes following the first and succeeding exposures are similar to those described in experiment 1. Normal or slightly higher levels are reached either within 5 hours or at most by the morning after the irradiation. The constituents remain at these levels, sometimes rising still higher but showing the usual drop on subsequent irradiation. The fourth irradiation in experiment 2 is accompanied by an increase in reds, Hb and hematocrit during the exposure, followed by a marked decrease in the afternoon (5 hours later) and a return to above normal the next morning (see fig. 1 *c*), the whites and platelets showing the usual decrease immediately after. The changes in specific gravity are directly proportional to those in the reds. Plasma volume as determined at the end of the exposures is increased almost exactly in inverse proportion to the decrease in the blood constituents. Thus, for example, in experiment 5, the reds and Hb are decreased 21 and 26 per cent respectively following the second exposure and the plasma volume correspondingly increased 21 per cent.

In all the experiments, reds are high on the morning following the last (fourth) exposure. In experiment 2 they are about a million higher and slowly return to normal three days later. This is followed by a secondary rise to a level one and a half million (26 per cent) above normal 7 days later which level persists for about two weeks and then slowly drops to approximately normal about six weeks after the last irradiation. In experiment 3 the last irradiation is followed by a decrease in reds to the normal level, a slow increase to 1 million above normal three days later, a drop to normal level, and a secondary rise to a level 1 million (17 per cent) above normal 12 days after the irradiation, which level is maintained for about 3 weeks. Similarly the last irradiation in experiment 4 is followed by an increase in reds of about 700,000, a decrease to normal 2 days later,

a secondary rise one week after irradiation and a return to normal; while in experiment 5 the reds are 1 million above normal after the last irradiation and remain at this level for 9 days. They then show a secondary rise to a maximum of 2 millions above the pre-irradiation level 3 days later, followed by a gradual drop to a level about 1 million above normal maintained until the end of the experiment (3 weeks after the last irradiation), the average level for the post-irradiation period being 19 per cent above that preceding irradiation.

The Hb changes in the first part of experiment 2 follow those in red cells quite consistently, with a resultant normal color index. Following the third irradiation, however, and throughout the post-irradiation period, although still about 3 per cent above normal, the increase in Hb is proportionately smaller than that in reds, thus leading to a low color index. The same effect was observed in the post-irradiation period of experiment 1. Hb in experiments 3, 4 and 5 also roughly follows the changes in the reds, again, however, showing smaller increases than the reds during the post-irradiation period and a resulting low color index, the average index for the post-irradiation period in experiment 5 being only 0.7. Similarly hematocrit and volume index roughly parallel the changes in the red cells during the irradiation period except for the period immediately after the last irradiation in experiment 3 when, with a normal red cell count and Hb, the hematocrit is low, rising to normal 3 days later. In both experiments 2 and 3 cell volume and volume index in the post-irradiation period are lower, which, coupled with the fact that the Hb is relatively low, results in a normal saturation index, i.e., the cells are smaller and the saturation per unit of volume has not changed materially. In the later part of the post-irradiation period in experiment 3 (2 weeks after the last irradiation) the secondary rise in reds is accompanied by a correspondingly high hematocrit but a low Hb, so that the saturation for the subsequent two weeks is only 65 per cent normal (see expt. 1). In experiments 4 and 5 the volume and saturation indices fluctuate within normal limits during the irradiation period. The saturation index in experiment 5 is low during the post-irradiation period due to the relatively low color index.

Platelet number and volume follow a course similar to that in experiment 1. The decrease on irradiation is consistently present. No definite increase was observed except in experiment 4 where the last exposure was followed by a rise of about 10 per cent, which increase was maintained for about 2 weeks. The whites again show a steadily decreasing level during the irradiation period. In experiment 2 the third exposure is followed by a 20 per cent increase which slowly recedes to below normal before the last irradiation, rising again to the same high level and remaining so for 10 days after, then dropping to 50 per cent of the original value at the end of 5 weeks. The first exposure in experiment 3 is followed by a 40 per

cent increase in whites, a decrease following the second irradiation and a return to the same high level on the next morning, followed by a steady decrease to a level 30 per cent below normal a week after the last exposure. This low level persists for about a week and returns to normal at the end of a month. In experiment 4 they show a steadily decreasing level during the irradiation period, the level 2 days after the third exposure being only 50 per cent normal and only 80 per cent normal 4 weeks after the last exposure. In experiment 5 whites show little change, rising after the last irradiation and quickly returning to normal.

The effect on the differential white count is again inconsistent. In experiment 2 the lymphocytes increase at the expense of the neutrophiles during the irradiation, although the opposite is also the case. In the post-irradiation period, the relative lymphocytosis is more definite. In

TABLE 2

Composite average of 4 experiments for pre- and post-irradiation periods

	REDS	Hb	COLOR INDEX	HEMATOCRIT	VOLUME INDEX	SATURATION INDEX	PLATELETS	TOTAL WHITES	LYMPHOCYTES	NEUTROPHILES	EOSINOPHILES
	millions	*grams per cent*		*per cent*			*in 1000's*		*per cent*	*per cent*	*per cent*
Pre-irradiation	5.58	10.48	1.00	35.4	1.00	1.00	274	10,760	23	71	6
Post-irradiation	6.13	10.50	0.83	35.8	0.89	0.95	272	10,930	22	73	5
Difference	+0.55	+0.02	−0.17	+0.4	−0.11	−0.05	−2	+230	−1	+2	−1
Per cent change	+10.00	+0.2	−17.00	+1.0	−11.00	−5.00	−0.7	+2	−4	+3	−17

the first part of experiment 3 there is an increase of large mononuclears with little change in the other forms, while in the post-irradiation period the neutrophiles are increased and the lymphocytes are relatively low. In experiment 4 the neutrophiles increase during and after the irradiation and return to normal at the end of the experiment. In experiment 5 lymphocytes increase after the second and third irradiations but decrease in the post-irradiation period, the changes in neutrophiles being in the opposite direction.

The values for the pre- and post-irradiation periods of the four experiments have been averaged and combined in table 2. The figures for the whites are deceptive as to the final low levels previously mentioned, since they include the high counts found in the first part of the post-irradiation periods. The principal effects of irradiation for experiment 2 have been collected in figure 2.

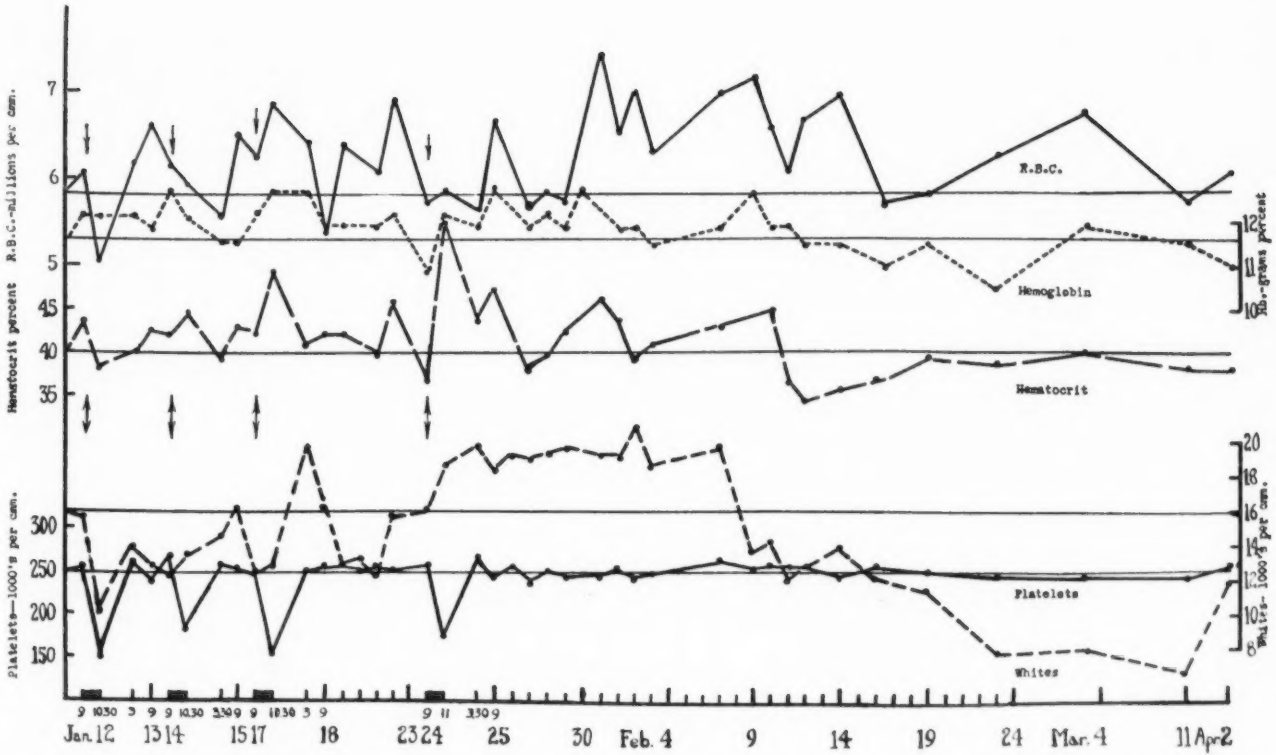

Fig. 2. Experiment 2. Arrows and heavy blocks show beginning and approximate duration of exposure. First three exposures are ½ hour at 60 cm. (67 g. cal. per sq. cm.). Fourth exposure is ¾ hour at 60 cm. (100 g. cal. per sq. cm.). Continuous horizontal lines show average pre-irradiation levels.

Experiments 6, 7 and 8. These experiments were performed primarily to follow in greater detail the volume changes during and immediately following irradiation using massive exposures. In experiment 6 the animal was irradiated 60 minutes at 60 cm. (133 g. cal. per sq. cm.) on two consecutive days while in experiments 7 and 8, the dose was 53 minutes at 80 cm. (67 g. cal. per sq. cm.) given in the morning and repeated again 3 hours later. Plasma volume determinations were made immediately before and after the first exposure, followed in a few days by a third determination. Specific gravity readings and counts in experiments 7 and 8 were made at 15 minute and half-hour intervals respectively.

A typical protocol is presented in table 3. The diffusion of water from the tissues begins almost immediately and proceeds rapidly so that at

TABLE 3

Experiment 8—blood changes following massive exposure

DATE	TIME	PLASMA VOLUME	TOTAL CELL VOLUME	SPECIFIC GRAVITY	REDS	HEMATOCRIT	Hb	WHITES
1927		*cc.*	*cc.*		*millions*	*per cent*	*grams per cent*	
Nov. 4	Before irradiation	648	244	1.0508	5.816	27.5	9.31	8,250
	After ½ hr. irradiation			1.0500	4.360	26.0	8.97	6,150
	After ¾ hr. irradiation			1.0458				
	After 1 hr. irradiation	834	208	1.0465	4.456	24.5	8.29	5,100
	½ hr. after irradiation			1.0488				
	3 hrs. after irradiation			1.0480	4.688	24.0	8.29	7,400
	After ½ hr. additional irradiation			1.0447	4.304	20.0	7.61	6,000
	After 1 hr. additional irradiation			1.0450	4.364	20.5	7.78	5,600
Nov. 5				1.0460	5.424	25.5	8.12	5,600
8				1.0465	5.424	25.5	8.63	5,700
10		774	309	1.0495	5.688	28.5	8.97	6,000

the end of half an hour the reds are decreased 20 and 25 per cent in experiments 7 and 8. After this the increase in plasma volume is more gradual. In experiments 6 and 7 the increase continues and is at a maximum 3 hours later, although in experiment 8 further irradiation after the first half-hour produces no greater increase and the volume has already begun to return to normal 3 hours later.

Plasma volume determinations at the end of the first hour of irradiation show increases of 28, 36 and 22 per cent, respectively, in the three experiments. The second irradiation is again accompanied by a comparable dilution but in all experiments there is evidence of slight concentration at the end of the hour. Corresponding changes in the specific gravity confirm these effects. With the exception of the whites, the values are all

approaching normal by next morning and, as in previous experiments, in some cases tend to rise above normal and remain so for a short while. In none of these experiments do the whites return to normal, remaining at levels 20 to 30 per cent below at the conclusion of the experiments (from 1 to 3 weeks after the last irradiation). In each case there is a decided lymphocytosis following the last exposure, being most marked in experiment 7 where the lymphocytes increase almost 300 per cent.

Our results are in close agreement with those of Miles and Laurens (1926) and also confirm the suggestion of Mayerson (1927) that the low values in blood serum Ca and P which he found immediately after irradiation are due to shift in blood volume rather than to an actual decrease in the constituents. As Barkus and Balderrey (1924) have also shown, the primary effect of irradiation is vasodilatation followed by diffusion of tissue fluid into the blood stream causing blood dilution and increased blood volume. If the irradiation is continued or repeated at short intervals the dilution may be counteracted by overheating, resulting in vasoconstriction and blood concentration. These findings, we believe, should facilitate the interpretaton of results obtained by various investigators. Thus Hardy (1927) obtained an initial drop in reds, whites and platelets on irradiation of rabbits with the quartz Hg vapor lamp with return to normal levels or above by the next day. Variation in the amount of irradiation had little effect on the extent of the drop, but the duration was proportional to the dose. Similarly Bannerman (1927) found that single massive irradiations of non-tuberculous orthopedic cases with the quartz Hg vapor lamp were always followed by decreases in the number of reds beginning immediately and lasting for several days. In some these were followed by increases above the normal pre-irradiation levels. These results, very similar to ours, are, we believe, to be interpreted on the basis of increased plasma volume.

Although the responses of the reds during the irradiation period may vary, there is a greater or less increase in the count during the post-irradiation period. (See also Gunn, 1926; Hardy, 1927.) The effects are too pronounced and lasting to be due to any other factor than the radiation. Traugott (1920) has shown that there is little shifting of red cell concentration in the blood stream, and, as indicated above, identical counts were obtained from different parts of the body. Furthermore, one of the control animals kept for over 3 months under the same conditions and subject to the same procedure, but not irradiated, showed no such effects. In some cases, especially after massive irradiations, the red cells are larger and less saturated than before, while they are small and pale during the post-irradiation period. This may be due to the fact that during the irradiation period there is a minimum formation of new cells, the system calling chiefly on its reserves, while in the post-irradiation period the bone

marrow has exhausted its store and is making new cells which are low in Hb and of a low color index. Effects such as these have frequently been observed in conditions where there is a strong stimulus to erythropoiesis, small pale cells in regeneration from hemorrhagic anemia (Whipple and Robscheit-Robbins, 1925) and in emotional polycythemia (Izquierdo and Cannon, 1928) and large unsaturated cells in severe anemia (Murphy, Monroe and Fitz, 1927).

The constancy of the decreases in the platelet count and volume during irradiation is outstanding (see fig. 2) and has been used as an index of the dilution. In two experiments the post-irradiation values remain high, results similar to those of Miles and Laurens (1926). (See also Sooy and Moise, 1926; Gunn, 1926; Hardy, 1927.)

Most of the animals showed a leucopenia in the post-irradiation period. Similar observations have been recorded by many observers (Laurens, 1928). Bannerman (1927) reports that following quartz Hg vapor lamp irradiation the number of whites may either increase or decrease, but that there is always an increase in polymorphs. In many cases he also observed a decrease in the number of mononuclears. Hardy (1927) believes that the effect of ultra violet radiation on the lymphocytes is not selective but is proportional to the total energy of far ultra violet present whatever its wave length. The effect on the polymorphs, on the other hand, is selective and is proportional, not to the total intensity, but to the intensity of the radiation in the region from 295 to 320 $m\mu$. This may aid in interpreting our apparently inconsistent differential results, since our animals were exposed to the unscreened radiations from the entire spectrum.

Traugott (1920) has shown that massive shifts of white cells do not occur without mechanical interference or inflammatory reaction, phenomena which can not explain the observed leucopenia since the condition persists long after any direct effect must have disappeared. If we regard the course of the platelets as an index to dilution, the leucopenia must be explained, at least in the present experiments, on grounds other than lasting blood dilution, for platelets are normal or above normal in this period. The similarity in white counts of blood samples taken from different parts of the body would discount any mobilization of the leucocytes, so that, as Reed (1925) points out, it must, for the present at least, be assumed that they were destroyed as a result of the irradiation. He suggests that the penetration of cells by rays of appropriate wave lengths activates oxidative processes and, if the acceleration exceeds certain limits, there is destruction and disintegration of protoplasm with consequent injury to the organism as a whole. The resistance of the red blood cells is in keeping with their metabolism. This may explain in part the leucocytosis on moderate irradiation and the leucopenia on stronger excessive

exposures which Miles and Laurens (1926) obtained. The above explanation, however, is insufficient to account for the lasting leucopenia and the changes in the other constituents unless we assume a concomitant formation of irradiation products which are absorbed by the blood and carried throughout the system and which in some way affect the organs responsible for the formation of red and white cells and platelets.

That red cells may also be destroyed by excessive irradiation is indicated in the experiments where massive exposures were used. Preparations made at the end of these periods present clear evidence that cell destruction is taking place. There is anisocytosis and poikilocytosis and much fragmentation. Levy (1924) and Hobert (1923) reported that short exposures to a quartz Hg vapor lamp stimulated red cell formation in regeneration from anemia, while too strong doses increased destruction. Ohta (1924) reported that sunlight decreased the number of red cells after sensitization of rabbits with hematoporphyrin. Many of the conflicting results reported in this field are without question due to the variation in the intensity and character of the radiation, the specification of which in comparable units is all important. Our experiments also show the necessity of taking into account changes in blood volume in the interpretation of results obtained. Since radiation acts as a hematopoietic stimulus to the normal relatively stable organism, it is a plausible notion that it would be particularly efficient in effecting regeneration in anemic conditions. Results of observations bearing on this point will be reported in a separate publication.

SUMMARY

The primary result of an individual exposure to C arc radiation is a temporary increase in plasma volume of 6 to 37 per cent with recovery to normal within 5 hours. This dilution reoccurs but is not augmented by further exposures, its duration, however, being determined by the strength of dosage and the interval between successive exposures. After massive exposures a slight concentration follows the initial dilution. Repeated exposures in five experiments stimulated the hematopoietic system as shown by increases in red cell number of from 10 to 19 per cent, maintained for from 3 to 6 weeks after the last irradiation. Color, volume and saturation indices show that the cells in the post-irradiation period are usually smaller and less saturated than before the irradiation. Platelets drop consistently in number and volume (35 per cent) during irradiation with recovery usually within 5 hours after each exposure and thus serve as an index of the dilution. In two experiments the levels are increased (4 and 15 per cent) and remain high for about 10 days after irradiation is stopped. There is a progressive leucopenia during irradiation which results in markedly low levels in the post-irradiation period.

We take pleasure in recording here our thanks to Miss Virginia Butler and to Miss Irene Sellier for technical assistance.

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QUANTITATIVE RECORDING OF THE KNEE-JERK BY ANGULAR MEASUREMENT

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As the knee-jerk is usually recorded by vertical lines with the kymograph, there is danger that the impression will be gained that equal divisions of a line always have an equal representative value. However, the uppermost centimeter of such a line may represent twice the angle of jerk represented by the lowermost centimeter or *vice versa* or some other relationship may obtain: we do not know simply from inspection of the graph presented to us. Much depends on how the apparatus is arranged for the purposes of registration; as a rule no attempt is made to secure constancy of mathematical relations from time to time with the same individual nor with various individuals whose limbs differ in length and no figures are presented to enable us to evalutate the variations. We should not expect the units of a straight line to have an equal representative value even if the line secured were the function of the sine of the angle of knee-jerk, since such a function varies at different rates as the angle changes from 0° to 90°; but in apparatus generally described, no attempt is made to secure any true mathematical function of the angle or arc of movement. For example, a string attached to the heel so that movements are recorded in the familiar manner fails to give a true chord of the arc of movement. To obtain a record free from the above objections requires a deliberate design of recording apparatus for this purpose. A step in the direction of standardization seems worth while. Accordingly we have sought to secure a graph which is a known magnification of a linear function from which can be calculated the magnitude of the knee-jerk in degrees as produced in the movement at the knee-joint.

The other chief aims of the apparatus here described are as follows: 1, to have the subject fairly comfortable, which is important if tests are to be protracted in time; 2, to prevent slight slips of position of the knee during a period of tests since, if the hammer strike at a different spot, there may arise a difference in extent of the jerk; 3, after each jerk to secure a return of the leg by its own weight to a position identical with or nearly identical with its position before the jerk, thereby producing a horizontal

or an almost horizontal base-line; 4, to cause the writing-point on the drum as accurately as possible to represent the true position of the leg at any moment, avoiding loss of motion and overshooting in the recording apparatus; 5, to permit the investigator to magnify the movement in the process of recording and to change the degree of magnification if he desires.

A comfortable Morris chair is elevated on 6 inch metal legs. The subject's left foot rests, if convenient, on a footstool, while his right leg reclines in the steel V-shaped support, *J*, shown in figure 1. *J* is attached in a longitudinal slot in a board, *H*, and its position is adjustable with a thumb-

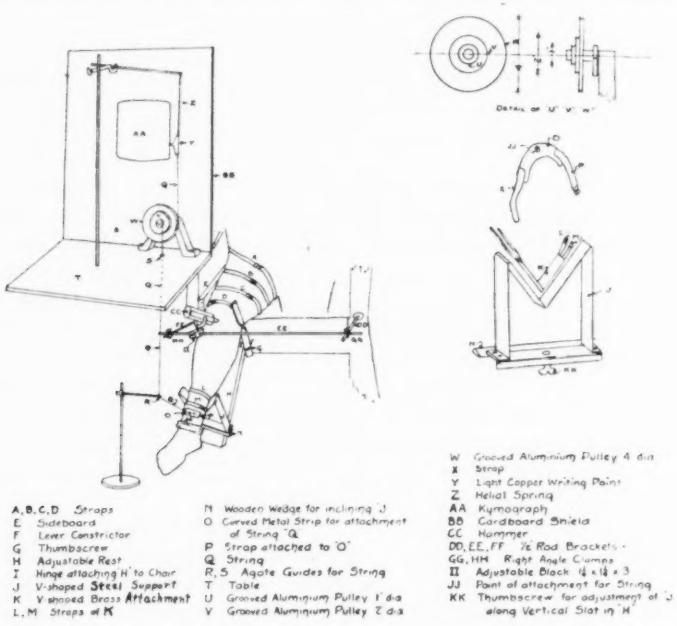

Fig. 1. Quantitative registration of knee jerk

screw, *KK*, while its inclination can be altered with a wedge, *N*. The angle of *H* can be changed by means of a hinge, *I*, and a pinion and rachet (not shown in the figure). Three straps, *A*, *B*, *C*, firmly fix the thigh to the sideboard, *E;* a fourth, *D*, is attached to *E* and to a metal strip, *F*, which by means of a thumbscrew, *G*, can be made to press the thigh toward *E*. A V-shaped piece of brass, *K*, fitting on the posterior side of the leg at a selected point not far above the ankle, is secured with straps, *L* and *M*. *K* is 6 cm. in width, fitting into *J* but exceeding the latter by 2 cm., in order to provide for overriding. The attachment of *K* to the leg serves

to lead the leg as it falls back by its own weight to a fairly constant position after each knee-jerk, thereby making it possible to secure a fairly horizontal base-line. To add to the subject's comfort and stability of position, a feather pillow is placed behind him, if necessary, and another is tightly packed between his left thigh and the left arm of the chair.

For tapping the tendon, we have used the electromagnetic hammer of Johnson (1927), but to prevent slight slipping from position, due to its own forces upon impact, we employ extra clamps. The hammer, *CC*, is provided with a half-inch supporting rod which passes through a bore in a steel block, *II*, and which can be firmly fixed in any desired vertical position by means of a bolt. *II* is bolted to a half-inch rod, *EE*, which is at-

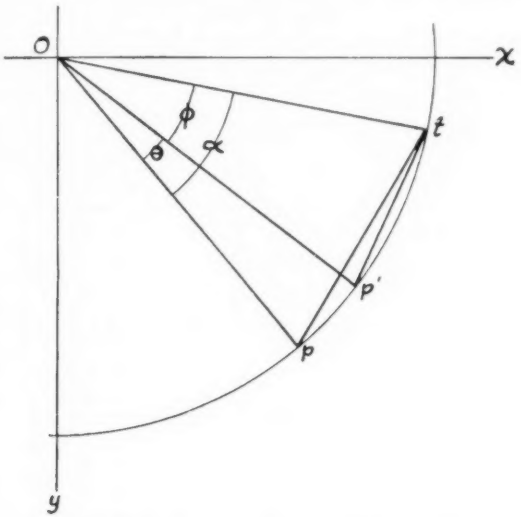

Fig. 2. Diagram showing geometric relations

tached with right angle clamps, *GG* and *HH*, to the brackets *DD* and *FF* fastened to the chair. Adjustment of the position of the hammer can be made laterally or by rotation by loosening *GG* and *HH*, permitting *CC*, *II* and *EE* to move as a unit. It would be desirable to take a series of measurements of the force of impact in order to learn what variation takes place in the stimulus. If desired, other means to strike the tendon, such as the patellometer of Pereira (1925), can be substituted.

In front of the subject is a table, *T*, supporting the recording apparatus which is concealed from view by a cardboard shield. A curved metal strip, *O*, is placed in front of the tibia and firmly attached to the leg a few centimeters below *K*. From *JJ*, a metallic tip on *O*, a stout silk

fishing cord, Q, runs through agate guides, R and S, to a point of attachment in the groove of the smallest of 3 pulley wheels made of a single piece of aluminum. The particular wheel represented in the figure makes it possible to secure magnification ratios 1, 2 or 4 as desired. Q runs further from the groove of the larger pulley wheel selected to a thin metal writing point Y which moves upward with each leg extension owing to a spiral spring, Z, making a vertical linear tracing on the slowly revolving drum for each knee-jerk. When the leg is at rest, Y draws a fairly uniform (but at any rate accurate) base-line.

TABLE 1

Showing the angles of knee-jerk (θ) as calculated for four subjects according to the formula given in the text

	SUBJECT	pt	op	$<poy$	α	VALUES OF g/n		
						Minimum	Average	Maximum
	D. M.	10.7	39	34°	16°	0.15	1.8	7.6
$p't$						10.55	8.9	3.1
ϕ						15° 32′	13° 6′	4° 36′
θ						28′	2° 54′	11° 24′
	I. B.	13.2	41.2	34°	18°	0.75	4.3	5.55
$p't$						12.45	8.9	7.65
ϕ						17° 22′	12° 24′	10° 42′
θ						38′	5° 36′	7° 18′
	M. F.	4.75	15	34°	19°	0.1	1.1	3.2
$p't$						4.65	3.65	1.55
ϕ						17° 50′	13° 48′	3° 36′
θ						1° 10′	5° 12′	15° 24′
	B. R.	12.5	38	40°	21°	0.02	.6	2.28
$p't$						12.5	11.9	10.0
ϕ						19° 10′	18°	15° 4′
θ						1° 50′	3°	5° 56′

At the beginning of each period, the table is placed so that the cord runs practically vertically, while the metal tip JJ coincides with the center of the agate guide R when the subject extends his leg sufficiently. Accordingly the mathematical relations become as shown in figure 2. Let op represent the line drawn longitudinally and medially down the anterior surface of the right leg from o, the assumed center of curvature, to p, the point the movement of which is to be calculated for each extension of the knee-jerk. For practical purposes o lies approximately at the mid-point of the patella, while p represents the metal piece, JJ. If t represents a point practically coincident with the agate guide R, and if g/n represents the length in centimeters of the graphic tracing, divided by the number of magnification, it is evident that $g/n = pt\text{-}p't$. From this $p't$ can be cal-

culated in centimeters, and using the formula $p't/op = 2 \sin \frac{1}{2} \phi$, since $p't$ is the chord of ϕ, the value of ϕ can readily be found from a table of natural sines. Finally $\theta = \alpha - \phi$, giving the desired angle of jerk. Accordingly it is necessary for a given experimental period to measure op and pt in centimeters and α in degrees. Then, knowing the value of any given g in centimeters, θ can be very simply determined. The experimenter may, if he desires, also measure the angle poy. Evidently the range of error involved in the above measurements should be calculated.

A specimen of angular measurements is shown in the following table. That linear graphs do not truly represent the magnitude of knee-jerk so as to permit comparison of those secured in different periods with one subject or with different subjects is clearly illustrated. For instance the linear graph, (g/n), of subject I. B. averages 4.3 centimeters, that is, approximately four times the average of M. F., but as shown by the corresponding values of θ, the actual average magnitudes of knee-jerk are approximately the same for the two subjects. Again, the ratio of the linear graphs (g/n) of the maximum jerks of D. M. and B. R. exceeds 3:1 but the ratio of the actual magnitudes (θ) of the same jerks is less than 2:1. The advantage of securing angular measurements therefore seems evident.

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STUDIES ON ADRENAL INSUFFICIENCY

VI. The Influence of "Heat" on the Survival Period of Dogs after Adrenalectomy

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We have already stated (Rogoff and Stewart, 1927) that the condition of heat enables the totally adrenalectomised bitch to survive much beyond the maximum period seen in control male dogs or females not in heat and non-pregnant. Data on one animal (record number 120–5) have been previously referred to (Rogoff and Dominguez, 1927). The effect of adrenalectomy on the blood pressure of the animal was being studied by the carotid loop method. The second adrenal was removed towards the end of oestrus. The animal survived about $36\frac{2}{3}$ days. The blood pressure was not affected by the condition of heat. Another bitch (121–9), in which the second adrenal was removed during heat, survived 32 days.

Condensed protocol. Dog. Record number 121–9. October 5, 1926, weight 9.1 kgm. Right adrenal (0.60 gm.) removed. October 15, 1926, weight 8.5 kgm. Left adrenal (0.62 gm.) removed at 10:30 a.m. Post-operative recovery excellent. Up to and including November 11, 1926, the animal remained in excellent health, eating well and very lively. On November 12 she seemed somewhat less lively and although she ate a good meal (meat) her appetite was not as keen as before. Refused biscuit absolutely. November 13, distinctly less alert than heretofore but not at all asthenic. Reluctantly ate a portion of meal (meat) and some rabbit. November 14, some bilious vomit. Refused food completely. Apathetic. November 15, getting more apathetic. Total anorexia. Perfectly intelligent. Somnolent. November 16, at 10:10 a.m., died without a struggle. Autopsy at once. Pancreas markedly congested. Stomach and whole of small intestine contained blood (and bile). The mucosa was congested extensively in the stomach and less so (in patches) in the small intestine. Uterus, non-pregnant, hypertrophic. Ovaries congested; numerous corpora lutea visible on surface.

Nothing in this protocol calls for special remark. The survival period was of course much beyond anything seen in the very extensive series of controls (at the time of writing, about 150). This is true of all of the four animals included in the paper. The period of good health extended, as in the great majority of the controls, up to 2 or 3 days before death. Acute symptoms apart from anorexia and slight emesis were absent. The sudden

change for the worse between the last night and morning with no acute symptoms, not even coma, is unusual.

The third dog (124–0) had the first adrenal removed as soon as she was observed to be in heat, while the bloody discharge was going on. Eleven days later, the second adrenal was excised. The fact that the beginning of the pro-oestrum is marked in dogs by this discharge renders them specially suitable for such experiments as those described. In dog 124–0 the second adrenalectomy was performed before heat was over but towards the end of it. The prolongation of life and of the period of health although not as great in dog 124–0 as in other dogs, was nevertheless quite distinct. The animal survived the loss of the second adrenal $21\frac{1}{2}$ days, and remained in good health and eating well for about 19 days.

Condensed protocol. Dog. Record number 124–0. November 5, 1926, weight 9.25 kgm. Right adrenal removed. November 9, weight 9.1 kgm. November 16, weight 9.35 kgm. Left adrenal removed at 10:15 a.m. Excellent recovery. November 17, condition excellent. November 18 and 19, took some food but not much. November 20 and 21, appetite improved. November 22 to December 4, 1926, her condition was very good. Appetite excellent. December 5, refused food. December 6, total anorexia; rather apathetic; not asthenic. December 7, refused all food; rapidly failing. At 4:00 p.m., quite asthenic and very somnolent. At 9:00 p.m., completely comatose. Pulse slow and feeble; respiration slow and shallow. Passed a small amount of bloody fecal matter. Died at 10:20 p.m. *Autopsy* at once. Pancreas greatly congested. Considerable amount of blood in stomach, small and large intestines; mucosa of whole gastro-intestinal tract much congested and hemorrhagic. Kidneys, liver and spleen not greatly congested. Ovaries somewhat enlarged; many corpora lutea. Uterus large, non-pregnant.

The fourth dog (124–8) was in some ways the most interesting of the series, particularly in the long survival period, about $64\frac{2}{3}$ days. The second adrenal happened to be removed shortly before the beginning of pro-oestrum. The changes accompanying or initiated by heat were sufficient to carry the animal on for a period longer than the average gestation period in dogs. The nutrition of the animal was well maintained, as shown by the body weights and post mortem condition.

Condensed protocol. Dog (record number 124–8). Weights on November 30, December 9, 17, 23, 31, 1926, January 10, 1927, January 17, 24, 31, February 7, 13 and 15 were 11.6, 11.4, 11.3, 11.55, 11.15, 11.1, 11.1, 11.1, 10.8, 10.8, 10.6 and 10.6 kgm. respectively.

November 30, 1926, right adrenal excised. February 15, 1927, left adrenal excised at 10:00 a.m. Excellent post-operative recovery. February 16 to 18, ate well, but not very active. February 19, good appetite. February 20, quieter, not eating much. February 21, condition excellent. Ate well. She showed signs of heat. Attracted male dogs. February 23, bloody discharge from vagina was observed. She remained in very good condition and eating well till March 7, when she seemed less alert. Refused bread and milk but took meat readily. No asthenia but not very active. March 8 to April 15, remained in good health, eating meat and bread and milk regu-

larly but sometimes refusing biscuit, which previously she ate readily. The weights on March 14, 21, 29, April 4 and 11 were 9.6, 9.5, 9.75, 9.9 and 10.1 kgm. respectively. April 15, she did not care for bread and milk now but ate only meat. April 16, seemed to be getting less active but ate meat readily. Eyes "mattery" from this time on. April 17, ate meat rather reluctantly but finished the meal. Decidedly less active. April 18, lay about a good deal. Could only be coaxed to take a few small pieces of meat. Not weak. April 19, apathetic, but not asthenic. Walked well when coaxed out of cage. Complete anorexia. Slight emesis. April 20, decidedly more apathetic. Asthenic. 5:30 p.m., heart slow, some emesis (bilious). Refused all food. April 21, died early in the morning.

Autopsy. Fat plentiful in usual situations. Liver, spleen and kidneys not congested. Pancreas, no congestion whatever. Little, if any, congestion in the gastro-intestinal mucosa except in two or three Peyer's patches. No blood anywhere in lumen. The fundus of stomach was invaginated into the pylorus. Right ovary larger than left, and showing prominent corpora lutea while the left showed no corpora lutea on the surface. Uterus somewhat thickened with endometrium more prominent than usual, forming folds. Certainly non-pregnant.

The very long period of survival after the second adrenalectomy is the most striking feature in this experiment. With the exception of occasional diminution of appetite lasting for a day or two and accompanied by some lessening of activity, the animal for almost the whole period was in good health. The serious terminal symptoms appeared about 3 days before death (anorexia, apathy). Decided asthenia was present only about 2 days before death. Emesis was never a prominent symptom. During the rest of the period of survival it would have been impossible to distinguish this animal from a normal bitch. She was extremely lively in the cage and romped around when let out of it, standing up in the cage to greet persons entering the room. During heat she exhibited the usual behavior, seeking out males, especially a totally adrenalectomised dog (127–4), whose second adrenal had been removed 3 days before. He responded readily but all possibility of impregnation was definitely excluded. The second adrenal was removed 77 days after the first, and about the beginning of heat. A point of interest is the absence of congestion in the gastro-intestinal tract and of blood in the lumen. This, however, is sometimes observed in the control dogs. More striking is the absence of congestion in the pancreas, which is rare in the controls.

Discussion. The results obtained on the four animals being uniform as regards the markedly increased survival period and the period of good health, we do not hesitate to conclude that the changes associated with heat do in some way compensate for the loss of the adrenals. The period of survival may even exceed slightly the average gestation period in the dog. When four animals are seen to survive the removal of the second adrenal into the 37th day, 32 days, into the 22nd day and into the 65th day respectively and these constitute the entire number of animals operated on in this condition, it is superfluous to multiply experiments. Nothing like

these results are to be found among a series of controls nearly 40 times as large. The longest period of survival was in a dog which lost its second adrenal about a week before the bloody discharge was observed. In the others the second adrenal was removed towards the end of oestrus. What the change is which is associated with the increase in the survival period it is not possible to say at present. One might think of the changes in the ovary connected with ovulation. The marked alterations in the uterine mucosa are not excluded as a possible factor, nor changes in the interstitial cells of the ovary. At present we have no experimental data on this question. Since we have established the marked influence of heat upon the survival period the similar influence of pregnancy (Rogoff and Stewart, 1927) must be considered from a new angle. Every pregnant dog, fertilized in the normal way, passes through a period of heat. Since heat in the absence of pregnancy is associated with an increase in the survival period, sometimes fully as great as the maximum seen in pregnancy, the question may be asked whether the apparent effect of pregnancy upon the survival period is not really the influence of heat. It would probably entail a great deal of work to settle this question if a sufficient number of experiments to be treated statistically were to be made. However, as the tissues affected in heat are for the most part the same as those affected in pregnancy, the changes being perhaps carried farther in the latter condition, it would seem probable that pregnancy, as such, has an influence in the same direction as heat. It may be remarked, and we have seen illustrations of this in our work, that pregnancy may sometimes be an unfavorable factor, adding to the handicap of loss of the adrenals. This is almost self-evident. The accident of the initiation of premature labor by the surgical operation itself (second adrenalectomy) has been observed by us. This does not necessarily diminish the chances of long survival. In the case of heat the condition does not add directly to the handicap imposed by adrenal insufficiency alone since heat causes such slight disturbance, at least in comparison with the formidable complications which may be introduced by pregnancy. The beneficial changes may be assumed to be present in every case of heat although the time of survival may vary with the position of the second operation in the heat period, and also, it is to be supposed, with the individual dog. The necessity of absolute exclusion of the possibility of impregnation is self-evident. In our animals this was done, and it was verified, post mortem, also that they were non-pregnant. The discovery that heat increases the survival period renders it necessary to use for controls only males, or females not in heat and not pregnant. The safest plan is not to use females at all. It is known that none of the females in our series of controls was pregnant, and it is practically certain that none of them were in heat. Only males are now employed by us as controls and this has been the case for a long time. Males are also being

used exclusively for studies of the effects of cortical extracts. It is possible, however, that the administration of such extracts from time to time to animals in heat (or to pregnant animals) might cause a still greater lengthening of the survival period. If this occurred and the difference was marked, or if a "picking up" of the animal by extracts after it had begun to decline could be distinctly made out this would also constitute a positive test of the efficacy of an extract. The fact must be emphasised that physiological changes apparently so slight as those connected with heat should be capable of counteracting the extremely serious, and indeed uniformly fatal effects of total adrenalectomy in dogs. All the indispensable factors formerly supplied by the adrenals must therefore be substituted for by other tissues or processes. While we make no statement as to the tissues involved, the suggestion that cells in the ovary, similar in origin and structure to the adrenal cortex are responsible is a plausible one. If these cells (or the uterine mucosa) contribute something similar to the active substance of the adrenal cortex during the periods of sexual quiescence, this contribution could hardly be expected to be a sensible one as compared with that due to the cortex. Nor can it be assumed without proof that even in heat these structures form or liberate appreciable quantities of the supposed active substance. It is quite possible that the condition of adrenal insufficiency is a necessary stimulus. In paper III (Rogoff and Stewart, 1927) it has been pointed out that metabolic changes associated with pregnancy, apart from changes in the group of tissues mentioned, may in some way supply deficiencies or neutralise toxic conditions due to the absence of the adrenals. The same thing may be true of heat. On all these points, however, definite knowledge is entirely lacking.

SUMMARY

"Heat" (in dogs) is associated with a marked lengthening of the survival period and the period of good health after removal of the adrenals. All the dogs studied gave positive results. One lived into the 22nd day after excision of the second adrenal; one lived 32 days; one lived into the 37th day, and one into the 65th day. Nothing like these survival periods has been seen in the far more numerous series of control dogs (about 150).

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STUDIES ON ADRENAL INSUFFICIENCY

VII. Further Blood Studies (Cholesterol and Calcium) in Control Adrenalectomised Dogs

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In paper II (table 1) of the series (Rogoff and Stewart, 1926) estimations are given of non-protein nitrogen, urea nitrogen, uric acid, preformed and total creatinine, amino-acid nitrogen, undetermined fraction of non-protein nitrogen, Cl and dextrose in the blood of adrenalectomised dogs not subjected to any treatment. Before and since that time determinations of cholesterol and calcium were made by us. The non-protein nitrogen showed a marked rise when the characteristic symptoms, especially refusal of food, developed. The increase became greater as time went on up to death. An increase in the urea nitrogen is chiefly responsible for the augmentation of the non-protein N, but the "undetermined fraction" also increases. There is no significant change in the uric acid and the amino-acid N, sometimes a relatively small increase in the creatinine. The increase in non-protein and urea N often precedes the development of serious symptoms. A concentration of the blood due essentially to an increase in the number and relative volume of erythrocytes can be detected in most cases somewhat in advance of the onset of symptoms. The change then goes on increasing till death. It was stated that in a few instances in which the serum calcium was determined it seemed to be somewhat increased with the onset of the symptoms. In table 1 of the present paper the results of cholesterol and calcium determinations in 12 dogs (cholesterol in the blood, calcium in the serum) are given. These were additional control dogs. They are mentioned in paper V of the series (table 2) (Rogoff and Stewart, 1928), but the chemical work was reserved for the present paper. Cholesterol was estimated by the Liebermann-Burchard reaction, always making duplicate determinations. Chloroform extraction was made in a Lieboff apparatus sometimes with the introduction of a slight modification. This consisted of the use of a plaster paris disc upon which the blood sample (1 cc.) was distributed and dried. The disc rested upon the absorbent paper disc usually employed in the extraction tube. A number of determinations carried out in this manner, with known quan-

tities of added cholesterol, yielded very satisfactory results. To avoid evaporation of water from the bath, also condensation moisture on the stoppers of the extraction tubes, a layer of paraffin oil was allowed to cover

TABLE 1

Cholesterol in blood and calcium in serum of adrenalectomised male dogs

RECORD NUMBER	DATE	CHOLESTEROL IN 100 CC. BLOOD	CALCIUM IN 100 CC. SERUM	RECORD NUMBER	DATE	CHOLESTEROL IN 100 CC. BLOOD	CALCIUM IN 100 CC. SERUM
		mgm.	*mgm.*			*mgm.*	*mgm.*
128-9	4–25	192	13.0	130-4	5–18	170	11.0
	4–28	185	14.0		5–22	140	12.0
	4–30	150	15.5		5–23	152	13.0
	5–1	183	16.2		5–24	154	12.8
	5–2	136	17.0	130-8	5–11	185	11.0
	5–4	171	19.5		5–21	160	11.2
129-8	4–23	133	12.4		6–3	92	11.0
	4–25	132	13.3		6–4	120	10.8
	4–28	86	12.5		6–5	154	11.0
	4–30	120	12.0	130-9	5–6	196	11.5
	5–1	80	11.5		5–25	177	11.2
	5–2	150	12.5		6–3	204	11.4
129-9	5–11	142	11.5		6–5	240	12.5
	5–13	160	11.5		6–8	242	14.6
	5–15	160	11.5		6–10	208	15.0
	5–17	133	11.5		6–11	240	15.0
	5–20	85	12.8		6–13	172	15.8
	5–21	115	12.5		6–15	196	17.2
130-0	4–26	212	11.2	131-0	5–9	195	11.5
	4–29	184	11.0		5–25	178	11.4
	5–1	166	12.5		6–3	184	12.1
	5–2	160	13.0		6–5	202	13.0
130-1	5–18	171	11.0		6–8	218	15.0
	5–22	160	10.5		6–10	218	16.0
	5–23	172	11.0		6–11	236	15.6
	5–24	150	11.0	131-3	5–17	150	11.0
	5–27	170	12.5		6–1	172	11.4
130-2	5–18	196	12.8		6–6	215	11.5
	5–22	175	12.5		6–8	185	12.6
	5–24	171	12.4		6–10	162	14.5
	5–27	175	14.0		6–13	170	15.4
	5–28	194	14.8		6–15	133	17.0
130-3	5–4	218	12.0				
	5–9	245	13.2				
	5–11	266	15.0				
	5–13		16.5				

the bath. Calcium was estimated by Kramer and Tisdall's method as modified by Clark and Collip (1925). The data relating the events in the history of the animals to the time of collection of the various blood speci-

mens are to be found in the condensed protocols following table 1. The adrenal operations were done in the forenoon. Unless otherwise stated the blood was taken at 8:00 to 9:00 a.m. The last meal was on the previous day about noon. In every case the bodyweight was well maintained throughout.

Dog 128-9. March 15, 1927 left adrenal and April 21 right adrenal removed. Quarantine (snuffles) March 21 to April 5. Excellent health till April 28, when hallucinations and convulsions developed. April 30, slight yelling fit. May 1, slight wobble in walking. Eating less (biscuit and some meat); refused meal of meat. May 2, better; ate fair portion of meat. Slight wobble in walking. Blood (dark) at 10:00 a.m. In evening the animal became apathetic. May 3, ate nothing, but is fairly active. Slight wobble. May 4, apathetic. Blood (dark) at 9:00 a.m.; slow flow. May 5. Unchanged. Total anorexia. Not asthenic, although slight wobble. May 6. At 10:30 a.m., unchanged. 3:00 p.m. to 11:00 p.m., worse. Some emesis (bile); asthenic. May 7, dead at 6:15 a.m.; still warm.

Dog 129-8. On March 23, 1927 right adrenal and on April 21 left adrenal removed. Excellent recovery. Body-weight on March 28, April 4, 11 and 25, 9.2, 9.3, 10.25 and 10.6 kgm., respectively. Health was very good till April 29 and 30, when barking spells developed, but appetite remained good. Short convulsion on April 30. May 1, quiet; ate little. May 2, convulsion (repeated 2 or 3 times) with coma. Total anorexia. Some emesis. Blood obtained at 10:00 a.m. just after a convulsion. May 3, emesis (bile) just before death at 8:45 a.m.

Dog 129-9. On March 23, 1927 right adrenal, on May 12 left adrenal removed. In quarantine with cough 2 weeks before second operation. Torticollis developed about 3 days after first operation and persisted till death. Health remained excellent till May 19; appetite good; occasional emesis. On May 19, ate little, but fairly active. May 20, decidedly less alert. Ate nothing. Not asthenic but slight wobble in walking. May 21, semi-comatose; very asthenic. Blood (dark) obtained at 8:00 a.m., very slow flow. Died at 9:45 a.m. Weights on March 23, 28, April 4, 11, 21, 25, May 10, 12 and 17, were 7.0, 6.8, 6.8, 6.6, 7.25, 7.3, 7.5, 7.5 and 7.45 kgm., respectively.

Dog 130-0. On March 25, 1927 left adrenal, and on April 26 right adrenal removed. Blood taken before second operation. Ate well till May 1; some emesis. April 29, a short yelling spell in the morning. At noon ate good meal (meat), and had another yelling spell. Later became quiet. Blood was taken at 10:00 a.m. On May 1 and till the end refused all food. At 8:30 a.m., good flow of blood during collection of specimen. May 2, apathy; asthenia; anorexia; emesis (bile). Semi-coma. Died 5 hours after the blood specimen was taken. Blood dark; slow flow. Body-weight well maintained.

Dog 130-1. On March 25, 1927 left adrenal and on May 19 right adrenal removed. Excellent recovery. Is a moderate eater and apparently has recovered usual appetite. May 22 to 25, unchanged. Ate fair quantities of meat but slowly. May 26, less alert. Ate very little (if anything). May 27. Refused all food. Somewhat apathetic. Not at all asthenic. Tarry stools. Blood dark; slow flow. May 28. Comatose. Died at 9:00 a.m. Weights on March 25, 28, April 4, 11, 21, 25, 30, May 10, 16, 19, and 22 were 7.05, 8.1, 8.1, 8.7, 8.9, 9.0, 9.6, 9.65, 9.6, 9.6 and 9.3 kgm., respectively.

Dog 130-2. Young animal. On March 25, 1927 left adrenal, and on May 19 right adrenal removed. Very good recovery. Till May 26, ate voraciously; was lively and pugnacious. May 26, getting less active. Ate little. May 27, decidedly less active;

refused all food. May 28, apathetic, quite wobbly. Blood (dark) specimen 9:00 a.m.; blood flow slow. At 4:00 p.m., emesis (bile). Apathetic and asthenic. May 29, dead in the morning. Weights on March 25, 28, April 4, 11, 21, 25, 30, May 10, 16, 19 and 23 were 6.9, 6.55, 6.7, 7.3, 7.85, 8.0, 8.3, 8.4, 8.5, 8.7 and 8.5 respectively.

Dog 130-3. On April 19, 1927 right adrenal, on May 5 left adrenal removed. In good health till May 11. Then began to get less active and to eat less. On May 12 refused food; emesis. May 13 beginning asthenia; apathy; emesis (bile). At 10:45 p.m., comatose. May 14, in the morning was dead. Weights on April 19, 25, 30, May 5 and 10 were 7.2, 7.3, 7.75, 7.9 and 7.75 respectively.

Dog 130-4. On April 19, 1927 right adrenal, on May 19, left adrenal removed. May 22, good condition, eating fairly well. May 23. Total anorexia; less active. May 24, apathetic but not asthenic. May 25. More apathetic. Eating nothing. 6:00 p.m., stupor. At 9:00 p.m., dead. Body-weight had been maintained throughout.

Dog 130-8. Left adrenal removed on May 12, 1927; right on May 31. Ate well on June 1 (biscuit). On June 2 ate biscuit readily. June 3 refused food. Blood specimen (dark); slow flow. June 4. Stitch abscess (drained); short barking fit. Took some biscuit but later refused it. Slow flow in getting blood specimen. June 5, apathetic; total anorexia; slow blood flow. June 6, unchanged. June 7, dead in morning. Abscess around some stitches, not penetrating peritoneum. Large ulcer in duodenum, not perforated.

Dog 130-9. Right adrenal removed on May 10, 1927; left on May 31. Condition very good up to June 13, when he began to refuse food. Ate very little meat. Blood sp. gr. 1,038 on June 10, 1,038 on June 13. June 14, somewhat less alert; refused all food; some emesis (bile). Head shakes slightly. June 15, slight wobble in walking. Total anorexia. Blood sp. gr. 1,042. June 16, dead at 7:30 a.m. Weights on May 10, 31, June 7 and 13 were 11.35, 12.1, 11.9 and 11.4 kgm. respectively.

Dog 131-0. Young dog. Right adrenal removed on May 10, 1927, left on May 31 (2:00 p.m.). Health very good till June 4, when he had a barking spell about noon (lasting 5 minutes). June 5 to 7, no more barking spells; appetite excellent, but on June 7 some matter in eyes which condition as usual persisted till the end. June 8, active; good appetite. Hair coming out (a common symptom at this stage). June 9. Unchanged. June 10. Total anorexia, which continued till the end. Emesis (bile). Short yelling fit (about noon, four hours after blood was drawn). Thereafter became quite playful again, but took no food. Sp. gr. of blood 1,040. Slight wobble in walking in afternoon. June 11, quite asthenic; very apathetic; considerable wobbling. At noon, semi-comatose. Died between 1:30 and 2:00 p.m. Weights on May 10, 23, 31 and June 7 were 9.85, 9.95, 10.35 and 10.0 kgm. respectively.

Dog 131-3. Left adrenal removed on May 17, 1927, right on June 2. Good health and appetite till June 14, although from June 10 on he is eating less than previously. Sp. gr. of blood on June 10, 1,046. On June 13, took a fair meal (meat); is very lively and in excellent condition. Sp. gr. of blood 1,045. June 14. Little change. Ate biscuit, but not as greedily as before; refused meat; some emesis. June 15, slight wobble; apathy; total anorexia; emesis (bile). This was in forenoon. In afternoon more emesis (bile); tarry stool. Blood sp. gr. 1,046. June 16, dead in morning. Weights on May 17, 23, 31, June 2, 7 and 13 were 12.95, 12.6, 12.7, 13.0, 12.75 and 12.15 kgm. respectively.

Of the twelve dogs in table 1 more than half showed a decided increase in the calcium commencing with the onset of the serious symptoms or occasionally somewhat preceding them (e.g., dogs 130-9 and 131-3). In

the dogs which did not show such distinct increases in the calcium as the majority there was nevertheless an almost constant tendency for the calcium to creep up. In two or three of the animals there was no change, even when blood specimens were obtained at a time when marked symptoms were present. Thus in dog 129-8, the last specimen was drawn just after a convulsion and less than 24 hours before death, but the calcium was not altered. In dog 130-8 also there was no change in the calcium content of the blood serum throughout the period of observation, and it was noted that in collecting more than one of the specimens the blood flow was slow and the blood dark. These specimens gave the same calcium content as those collected with a normal flow. It must be remembered that the blood specimens were generally drawn at times predetermined by the routine and it was not practical to make the intervals between successive specimens very short. It is possible that sometimes a specimen taken nearer the end might have shown a positive result although the last one was negative.

Our early results, already alluded to although not included in table 1, are practically the same as the later ones.

Thus, in dog 102-5 the right adrenal was removed on April 30, 1925 and the left on June 5, 1925. The calcium on June 4 was 12.5 mgm.; on June 8, 11.6; on June 11, 14.3; on June 12, 15.2; and on June 14, 17 mgm.

In dog 103-7 the left adrenal was removed on June 3, 1925 and the right on June 12. The calcium was 11.0 mgm. on June 11 and 14 mgm. on June 15.

In dog 103-8 the left adrenal was excised on June 3, 1925, the right on June 10 (in afternoon). The calcium on June 10 was 11.2 mgm. (Blood drawn in forenoon.) On June 13 the calcium was 11.5 mgm.; on June 16, 12.5 mgm., and on June 18, 15.7 mgm. Conductivity measurements towards the end (on June 16) showed concentration of the blood. This was also the case in dog 103-7.

Determinations of calcium were made on two pregnant dogs.

In 103-6 the left adrenal was removed on June 3, 1925; the right on June 12. The calcium on June 11 was 11.5 mgm., on June 15, 13.4 mgm.; on June 19, 14.3 mgm.; on June 23, 16.5 mgm.; on June 26, 13 mgm.; on July 9, 15.7 mgm. On July 28, in the forenoon, the animal was comatose and received an injection of Ringer's solution at 11:00 a.m. Parturition began at end of injection but she was unable to care for the three pups that were delivered during the afternoon. Died at 5:30 p.m.

In dog 103-4 the left adrenal was removed on June 1, 1925, and the right on June 9. On June 8 the calcium was 10.7 mgm.; on June 13, 12 mgm.; on June 19, 12.5 mgm.; on June 23, 12.5 mgm.; on June 26, 11.6 mgm. On July 3 a litter of pups was delivered. On July 6 the calcium was 14 mgm; died today.

Data on the conductivity of the blood and serum, the concentration of the blood, blood counts, etc., are given in previous papers (Stewart and Rogoff, 1925; Stewart, 1926), on dogs 102-5, 103-4, 103-6, 103-7 and other dogs.

Discussion. We are not in a position at present to suggest any explanation of the relation between the hypercalcemia, if it may be so denomi-

nated, and the metabolic or other changes associated with adrenal insufficiency. It is not due merely to concentration of the serum, for it is the blood which is concentrated, not the serum. The blood has a much higher specific gravity and a much lower conductivity than before the consequences of the second adrenalectomy have developed. The specific gravity and conductivity of the serum remain unaltered within the normal limits of variation. The fact is illustrated in some of the protocols (e.g. dog 129-8) that blood specimens collected immediately after the development of very striking symptoms such as convulsions, or when the circulation is greatly slowed, may show no significant variation in the calcium content so long as the animal continues to eat and the disturbance is transient. This might be regarded as an indication that the increase in calcium seen in the majority of the animals commencing simultaneously with or somewhat in advance of the onset of the characteristic symptoms of adrenal insufficiency is no superficial phenomenon but like the increase in non-protein nitrogen, etc., a part of the picture of deranged metabolism induced sooner or later by loss of the adrenals. As to the relations between known chemical changes and symptoms, any discussion would be pure speculation at present.

As to the cholesterol, we do not think that any constant change related to the clinical condition of the animals or the onset of characteristic symptoms of adrenal insufficiency can be demonstrated from the results in table 1. If in some of the experiments there may seem to be a diminution in the cholesterol on the later dates, in others, and perhaps in the best and most complete series of observations (e.g., dog 130-9) this is not the case. If a conclusion were to be risked it might be that in fully half the cases the cholesterol tends to diminish rather than to increase as the interval since the second adrenalectomy increases. Yet it would not be possible to trace such an inverse relationship between the cholesterol and calcium contents in some of the most complete experiments (130-9 and others). We, therefore, prefer to draw no conclusion from the cholesterol determinations in table 1. They do not support the view brought forward by certain writers, often based on histological observations only, that the adrenals are peculiarly related to cholesterol metabolism. Results obtained by some observers on animals which only survived removal of the adrenals for a few hours or even a day or two, because of the inadequacy of the surgical technique, cannot be considered as reflecting changes associated with adrenal insufficiency.

SUMMARY

The calcium content of the blood serum of adrenalectomised dogs was generally found increased at the time of or sometimes a little preceding the development of the serious symptoms, especially anorexia, which terminate the period of good health.

The results on the cholesterol content of the blood do not permit us to conclude that any decided change in either direction is present either during the period of good health or after development of the terminal symptoms.

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PENETRATION OF ULTRAVIOLET RAYS THROUGH CLOTHING MATERIALS, PART II

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It was suggested in a previous paper (1928) that there are factors other than the fiber which may influence the transmissibility of ultraviolet rays through clothing materials. Recently evidence was presented by Hess et al. (1927), tending to show that the protection from sunburn afforded the skin by fabrics depends primarily upon the percentage of interspace due to weave. The U. S. Bureau of Standards (1927) reports measurements on the close- and open-weave fabrics to determine the relative transmission of ultraviolet radiations which each fabric admits after eliminating the light transmitted by interspace. The conclusion reached indicates that it makes but little difference whether the thread is of cellulose acetate, cotton, wool or silk, and that the importance of the composition of the material has been over-estimated.

Since the results previously reported were determined entirely by the biological effect of cottonseed oil irradiated through a clothing filter on the rachitic animal, it seemed pertinent to repeat this criterion to determine the influence of weave and weight of the fabric on its transmissibility of ultraviolet rays. The purpose of this paper is to report the findings from such investigation.

EXPERIMENTAL. In order to obtain materials of as nearly the same weave and weight as possible, approximately the same weight threads of cotton, wool, and artificial silk were woven on the same loom by the Union Knitting Mills at Logan, Utah. It was impossible to obtain linen and real silk threads. A description of the materials used is given in table 1.

The experimental procedure was practically the same as previously reported (1928). However, in this study the animals were placed in individual cages in order to obtain a record of their weights. The amount of fat was also changed from 15 to 10 per cent irradiated cottonseed oil.

Thirty-two rachitic rats were placed in seven test groups and one control

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group. Each group, except the control, was given a diet consisting of 90 per cent diet 3143 and 10 per cent cottonseed oil which had been exposed for 5 minutes through a clothing filter to the rays of a Hanovia A. C. air-cooled quartz mercury vapor lamp run at 220 volts and 2.5 amperes.

The ultraviolet-ray transmissibility of the material was determined by the rachitic potency of the irradiated oil. The test groups, as shown by table 1, were as follows:

Group I. Artificial silk, no. 1
Group II. Artificial silk, no. 2
Group III. Cotton no. 1
Group IV. Cotton no. 2
Group V. Worsted
Group VI. Worsted (50 per cent), cotton (50 per cent)
Group VII. Worsted (50 per cent), artificial silk (50 per cent)

TABLE 1

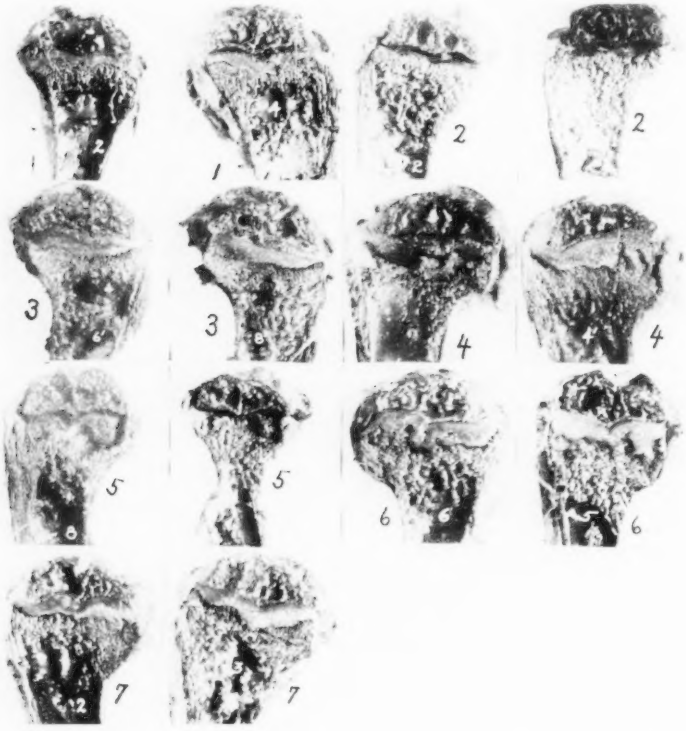
Description of the materials used

TEST GROUP NUMBER	FABRIC	FIBER	DESCRIPTION OF THREADS	NUMBER OF THREADS PER CENTIMETER	MEAN INTERSPACE BETWEEN 2 THREADS	WEIGHT OF A 4 BY 4 INCH PIECE	PERCENTAGE ASH
					mm.	*grams*	*grams*
I	Artificial silk (1)	Viscose	One 150 denier	30	0.584	1.3604	0.130
II	Artificial silk (2)	Viscose	Two 150 denier	18	0.169	2.5262	0.281
III	Cotton (1)	Cotton	One thread 2/70	23	0.240	1.3726	1.04
IV	Cotton (2)	Cotton	One thread 9 single 12	17	0.105	3.5732	1.175
V	Worsted	Wool	One thread 2/32 worsted	14	0.047	3.480	0.615
VI	Cotton (50 per cent); Worsted (50 per cent)	Cotton-wool	1/40 cotton, 1/40 worsted	18	0.146	2.9624	1.080
VII	Artificial silk (50 per cent); worsted (50 per cent)	Wool-viscose	2/32 worsted, 150 denier	13	0.238	2.5844	0.557

At the end of eight days the animals were etherized and the McCollum line test made, the results of which are shown in the accompanying reproductions of photomicrographs, representing typical tibial bones from each group.

Discussion. The tibial bones of group I show narrow proliferative zones of cartilage. In every case the cellular organization is regular, and the osteoid cells are well defined by the blackened calcium deposits. It

should be observed that this artificial silk filter has the least weight and the greatest mean interspace of the materials. The tibial bones of group II present a very similar picture. The filter in this case is also artificial silk but is made of two 150-denier threads, thereby increasing the weight and lessening the mean interspace. This difference seems not to have altered the ultraviolet-ray transmissibility of the filter. As shown by the photo-



Figs. 1–7. Photographs of the tibiae of two rats from each test group, I to VII inclusive.

micrographs, the oil irradiated through artificial silk filter induces an unequaled healing of the rachitic metaphyses.

Judging from the interspace and weight of cotton no. 1, it would be expected that the tibial bones of group III would show the same degree of healing as the tibial bones of groups I and II. That this is not the case is shown by the wide proliferative zones of cartilage. The tibial bones of group IV show no degree of healing. The inference is that if the cotton

material is heavy and tightly woven it is as impervious to ultraviolet rays as is worsted material. It is to be noted that the materials containing cotton have a high percentage of ash.

The tibial bones of groups V, VI and VII are of special interest because the clothing filters used contained worsted (see table 1). In group V the tibial bones show only a slight degree of healing; the same is true of the tibial bones of group VI; while the tibial bones of group VII show that healing had taken place, as evidenced by the comparatively narrow proliferative zones of cartilage and an undisrupted cellular organization. The worsted material has the least mean interspace; this measurement is not entirely accurate because the protruding hairs of the fiber lessened the interspace, and this reduction could not be measured.

CONCLUSIONS

From the data presented, it would seem that the mean interspace between threads and weight are factors limiting the ultraviolet-ray transmissibility of clothing materials. This conclusion is in accord with the results of previous workers who have quantitatively measured the light admitted by open- and close-weave fabrics. However, that weave and weight are the only factors is negatived by the percentage of ash, as shown in table 1. This fact strongly suggests that the porosity of the fiber is a significant factor which influences the ultraviolet-ray transmissibility of clothing materials.

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THE VARIATIONS OF THE WATER CONTENT OF THE BLOOD INDUCED BY ATMOSPHERIC TEMPERATURE CHANGES IN NORMAL AND SPLENECTOMIZED ANIMALS

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In a series of valuable studies of heat regulation and water exchange, Barbour and his associates have shown that both in laboratory animals and man, exposure to warmth is followed within a few minutes by dilution of the blood, and exposure to cold is followed by concentration of the blood. In connection with other studies we have repeated certain of Barbour's experiments and can confirm his statements that exposure of dogs and cats to cold and to ether anesthesia causes within twenty to thirty minutes a distinct increase in the percentage of blood solids, and that exposure to heat is followed by a decrease in blood solids and increase in the percentage of water.

Barbour has also cited evidence that the changes in blood concentration following exposure to atmospheric temperature changes are principally due to an exchange between the capillaries and the skin, subcutaneous and muscular tissues. That these changes are not primarily due to a discharge of corpuscles from the spleen, or to an epinephrin effect was inferred (2) from the fact that the sectioning of the splanchnic nerves did not prevent concentration or dilution of the blood on subsequent exposure to cold or warmth.

The recent work of Barcroft and associates (3) in which they have described very striking changes in the volume of the spleen of dogs under various conditions of emergency suggested to us a reëxamination of the question of whether or not changes in blood volume due to variations of the volume of the splenic reservoir, played any essential part in the regulation of the changes in the water content of the blood described by Barbour. Cannon and his associates (5) have given experimental evidence that exposure of animals to cold stimulates the adrenal apparatus which may secondarily stimulate splenic contraction with an outpouring of blood cells into the circulation (4).

In order therefore to determine whether or not the changes in blood concentration associated with temperature regulation against atmospheric cold and warmth are primarily exchanges between the blood and tissues

and not simply due to variations in the splenic output, we have studied the changes in blood concentration in cats following exposure to atmospheric cold and warmth before and after splenectomy.

Adult fasting cats were used throughout these tests. Twenty to twenty-five drops of blood were drawn from the marginal ear vein into a small weighing bottle and dried at 103°C. for 48 hours. All determinations were run in duplicate and results not agreeing to within 0.25 per cent were discarded. Preliminary control determinations of blood solids in which the blood samples were desiccated *in vacuo* over phosphorus pentoxid

TABLE 1

Changes in the water content of the blood of normal and splenectomized cats when transferred for fifteen to thirty minutes from a cool to a hot environment; and from a hot to a cool environment

+ indicates the per cent increase of the water of the blood (dilution) and − indicates the per cent loss of water from the blood (concentration).

PER CENT WATER IN THE BLOOD OF NORMAL CATS			PER CENT WATER IN THE BLOOD OF SPLENECTOMIZED CATS		
In the cool incubator	After exposure to the hot incubator	Per cent change of water content of the blood	In the cool incubator	After exposure to the hot incubator	Per cent change of water content of the blood
82.53	83.26	+0.73	83.10	83.73	+0.63
83.91	84.51	+0.60	84.22	84.80	+0.58
82.69	83.46	+0.77	83.26	83.45	+0.19
82.94	82.39	−0.55	84.53	85.26	+0.73
83.35	84.09	+0.74	84.45	85.35	+0.90
82.44	83.39	+1.45	81.45	81.63	+0.18
In the hot incubator	After exposure to cold incubator		In the hot incubator	After exposure to cold incubator	
84.15	83.79	−0.36	84.65	83.81	−0.84
81.72	81.48	−0.24	86.22	85.75	−0.47
84.54	83.62	−0.92	82.74	81.91	−0.83
83.89	82.34	−1.55	83.38	82.67	−0.71
			81.18	81.11	−0.07
			84.28	82.91	−1.37

agreed to within 0.1 per cent of the results obtained by drying in the constant temperature oven, hence the latter method was used in most of the work here described. The animal was put for 15 to 30 minutes in a large incubator at a temperature of 45° to 50°C. until polypnea or sweating appeared and then promptly removed and blood samples drawn at once for determinations of water and solids. For exposure to cold, the animal was put in a box on a piece of ice for 20 minutes and blood samples drawn immediately afterward. After these preliminary determinations, the cats were splenectomized and after one to four weeks the same procedures

were repeated. The results of the two sets of determinations are given in table 1.

It will be seen from this table that in both normal and splenectomized cats exposed for a few minutes to heat, there is an increase in the percentage of water in the blood (dilution) and that after exposure to cold there is a decrease in the water content of the blood (concentration). Furthermore, that the magnitude of the changes is similar in the normal and splenectomized animals. These changes of blood concentration are therefore primarily independent of the spleen although it may be that in normal animals, under certain conditions the emotional polycythemia described by Cannon may complicate the blood picture.

SUMMARY

We confirm the findings of Barbour and his associates that the exposure of laboratory animals to atmospheric warmth is followed by dilution of the blood and exposure to atmospheric cold is followed by concentration of the blood. Furthermore, the same changes of similar magnitude occur after splenectomy, indicating that these changes of the water content of the blood in response to atmospheric temperature changes are not due primarily to addition or withdrawal of blood from the splenic reservoir.

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THE EFFECT OF LIGATION OF THE COMMON BILE DUCT UPON THE APPEARANCE OF TETANY IN THYRO-PARATHYROIDECTOMIZED DOGS

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Dragstedt, Phillips and Sudan (1923) attribute certain detoxifying functions to the liver, endothelial cells and parathyroids, and suggest that the liver and endothelial cells by their detoxicating action may be the compensating factor in enabling completely thyro-parathyroidectomized dogs to recover from tetany.

In some work reported by Blumenstock and Ickstadt (1924) an attempt was made to determine whether or not the liver could detoxify intestinal toxins in the absence of the parathyroids. They showed a delay in the appearance and a diminution in the severity of the symptoms in dogs with the Eck fistula and thyro-parathyroidectomized as compared to thyro-parathyroidectomized dogs without Eck fistula. They concluded from these results that the rôle of the liver as the detoxifying agent in tetany seems to be questioned.

Later Blumenstock, Tweedy and Brannon (1926) in additional observations on Eck fistula thyro-parathyroidectomized dogs, reported findings that led them to conclude that the syndrome following parathyroidectomy is markedly changed in these animals. Their results show that factors other than the level of the plasma calcium are responsible for the symptoms following parathyroidectomy.

E. Gilbert (1925) recognized a new function of the liver when he discovered that the bile excreted two-thirds as much calcium as that excreted by the urine. The concentration of calcium excreted by the bile does not depend upon that excreted by the urine, but is a distinct function of the liver.

Assuming that the liver excretes about 66 per cent as much calcium as is excreted in the urine, and considering the importance of the calcium in the relief of parathyroid tetany, any method preventing this biliary loss should be of value. To determine what effect the conservation of calcium by biliary retention would have on the character and onset of parathyroid tetany, the common bile duct was ligated simultaneously with removal of

the gall bladder, thyroids and parathyroids in three animals. In a second group of five animals a similar operation was performed but the gall bladder was left intact. They were fed daily 400 to 500 cc. of milk, 125 to 150 grams of dog biscuits and two times per week 200 to 250 grams of ground meat.

Blood was drawn before and after operation for serum calcium estimations which were made according to Collip and Clark's modification of the Kramer and Tisdall method (1925). The blood was obtained either from the saphenous vein or heart. On the second or third day following the operation most of the animals showed some signs of toxemia and depression. Jaundice appeared on the first to the third day in the animals of the first group, but in those of the second group in which the gall bladder was not removed, jaundice did not appear for two to four days. The stools became clay colored on the second day in all the animals. Tetany was delayed in all cases and in some did not appear at all. Serum calcium increased 1 to 3 mgm. above normal during the first three days and decreased to the tetanic level on the fourth or fifth day. In some cases in which the calcium was below the tetanic level, no tetany appeared while in others there were mild symptoms of tetany with the serum calcium normal. The average length of life for the test animals was 8.44 days. Five animals had no tetany. Mild tetany appeared in the other three on the second, third and fourth days respectively.

Discussion. The results obtained would seem to indicate that the liver is concerned with the pathogenesis of tetany. The fact that tetany was delayed and in some cases did not develop at all would lead us to believe that the prevention of calcium excretion is the principal factor involved. The initial increase in serum calcium in some cases after total removal of the parathyroids indicates that at first there is a lessened excretion of calcium from the body.

Investigators have obtained different blood calcium values both in experimental and clinical jaundice. Buchbinder and Kern (1927) noted in puppies a persistent decrease in the serum calcium following ligation of the common duct with or without cholecystectomy. They suggested that the low calcium may be accounted for partially by the fact that growing bones use much calcium. Snell et al. (1925) and Walters and Bowler (1924) found practically no change in blood calcium in jaundiced dogs. The latter observed that it required approximately twice the amount of injected calcium to produce the same change in blood calcium in jaundiced dogs as in normals. King and Stewart (1909), King, Bigelow and Pearce (1911) and Lee and Vincent (1915) demonstrated an increase of calcium in the blood in obstructive jaundice.

In this report the parathyroids were removed simultaneously with ligation of the common bile duct and the animals did not live as long as when the

bile duct alone was ligated. However, they all lived sufficiently long to demonstrate that prevention of biliary excretion delayed and in some cases prevented the onset of tetany. Just how much delay in the onset of tetany was occasioned by the depression and intoxication from the absorption of bile cannot be stated.

As a possible contributing factor in causing delay of tetany in the animals here reported, the work of Roe and Kohn (1927) should be considered. They found that bile renders calcium less absorbable because of its alkalinity and content of fatty acids. The fatty acids precipitate calcium salts and form insoluble calcium soaps. The absence of bile in our animals may be an important factor in the increased absorption of calcium from the intestinal tract which would aid in the prevention of tetany.

The delay in the appearance and the amelioration of the symptoms of tetany presents a picture similar to that observed by Blumenstock et al. in the Eck fistula thyro-parathyroidectomized dogs. The late development or absence of tetany in both the Eck fistula and jaundiced animals may be due to the inability of the liver to eliminate calcium from the body, or it may be due to a change in the excitability of the nervous system from the depression occasioned by the bile salts and pigment.

In more recent work Buchbinder and Kern (1927) thyro-parathyroidectomized jaundiced animals and found marked amelioration of tetany symptoms. I obtained results similar to these in four thyro-parathyroidectomized animals after the common bile duct had been ligated for seven to ten days. The onset of tetany was delayed, the symptoms ameliorated in severity and in two cases no tetany developed. The average length of life for this series was 25 days after parathyroidectomy.

SUMMARY

Ligation of the common bile duct with or without cholecystectomy prevents the appearance of typical parathyroid tetany in dogs. Mild tetany may occur, and if it does it is delayed in onset and ameliorated in severity.

This delay in the appearance of tetany may be attributed to one or all of the following factors: *a*, a lessened excretion of calcium from the body by way of the bile; *b*, increased absorption of calcium from the gut because of the absence of the alkaline bile acids, which precipitate calcium; *c*, depression of the nervous system by the absorbed bile.

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THE SEASONAL VARIATION IN BASAL METABOLISM

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The basal metabolism data accumulated by the Nutrition Laboratory on normal individuals indicate that with any given adult, when basal conditions have been suitably recognized, the basal metabolism on any one day remains singularly constant from hour to hour (Benedict and Carpenter, 1918a) and the metabolism measured on the same person at different intervals over a period of time is not significantly changed unless there is a profound alteration in body weight or until after the age of forty (Benedict, 1928a). The evidence in connection with these data is sufficient, however, to suggest a possible seasonal variation in metabolism. Until recently basal metabolism measurements on the same individual have rarely been obtained at frequent enough intervals to enable determining whether there is a variability due to season. But what few records of this nature are available have thus far been confined almost exclusively to physiological laboratories, and fortunately deal for the most part with presumably normal people.

Eijkmann (1897), whose early researches are today receiving special attention because of their bearing upon the new interest which has arisen in the possible effects of race and the tropics upon metabolism, was the first, we believe, to study the influence of time of year upon the metabolism. He found the oxygen consumption exactly the same in winter and in summer. Lindhard (1910) noted a variability in his own respiration during a stay of two years in Greenland, which he was inclined to ascribe to the difference in sunlight in the dark and light periods of the year. In 1912 Lindhard published an extensive report dealing chiefly with the mechanics of respiration, but which likewise includes observations on the oxygen consumption of two men over a considerable period of time. Lindhard's deductions are that in the case of one of these men the lowest values for oxygen consumption occurred in January, February and March, the highest in April, November and December, and for the summer months the values were but slightly lower. With the other subject the consumption of oxygen rose regularly from February to August and the succeeding fall was less

regular, as the September experiments gave a strikingly low value. In January an appreciable rise took place, which he ascribes to previous diet. The varying intensity of sunlight is emphasized by Lindhard as being one of the greatest factors in the seasonal periodicity in respiratory functions.

Van Hoogenhuyze and Nieuwenhuyse in 1912 reported that the basal metabolism from November to February, with an average environmental temperature of 13°C., was identical with that from May to November with an average temperature of 23°C., and hence conclude that season is without significance.

It has been pointed out by Benedict (1915) that in the study reported by Palmer, Means and Gamble (1914) the six daily observations on Palmer from July 21 to July 26 gave values considerably lower than those noted during the preceding winter. Thus, in the summer the total heat production was 1797 calories and in the winter 2004 calories, with a body weight essentially the same.

Benedict and Carpenter (1918b), discussing the use of average basal values for comparison, touch also upon the question of possible seasonal variation in metabolism and record the oxygen consumption of 14 different subjects studied during a period of at least $7\frac{1}{2}$ months, the longest period being $6\frac{1}{4}$ years with one subject. They conclude that the metabolism of these subjects, as indicated by oxygen measurements alone, does not show regular seasonal variations but point out that there is a tendency for the high values to appear in the month of March and for the minimum values to occur in July. With students at the Y. M. C. A. College at Springfield, Massachusetts, the Nutrition Laboratory noted that the basal metabolism of the control squad B, when on normal diet, was perceptibly reduced between October and January (Benedict, Miles, Roth and Smith, 1919).

Young, at Townsville, Australia, reported in 1920 experiments made on himself and an associate with the object of studying the changes in respiratory metabolism under different atmospheric conditions. Although it is specifically stated that the results were not obtained with the subjects in the post-absorptive condition, he lays emphasis upon the influence of environmental temperature and concludes that the metabolism was greater in the warm season than in the cold season, a finding quite in accord with the observations of Osborne (1910). He explains the high level of metabolism as being due to a definite increase in body temperature produced by even slight exertion during the hot season, an increased body temperature which only gradually returns to a lower level after the exertion has been completed.

Smith (1922), studying in the Nutrition Laboratory the effect of severe muscular work, reports a long series of basal metabolism measurements with subject E. D. B., standing. He found with this subject a distinct tendency for the metabolism to be higher during the spring. Thus, the

level from January 2 to April 15 was perceptibly higher than the level between October 4 and December 23. Since, however, this series of observations involved a period of training and a considerable change in the subject's life, Smith is inclined to ascribe this increased metabolism in part to an increase in body weight and in part to a generally improved physical condition.

The longest series of experiments on one individual in which the influence of season can be studied is that of Kunde (1923), whose basal metabolism was measured practically every day for one complete year. In summarizing her seasonal variations in metabolism, for some unaccountable reason the author disregards the measurements between September and February, inclusive. She concludes that her metabolism during July and August was slightly lower than it was during any other month of the year. Since, however, the data for this subject show that her metabolism during the last half of a 15-day fast was at the same level as it was prior to the fast (an experience contrary to all other metabolism observations during fasting), one regrets extremely that the metabolism measurements could not have been made by another technician.

Collett and Liljestrand (1924), in studying the minute volume of the heart, report measurements of the oxygen consumption on the same subject covering a period of several months. They conclude that there was a rise in oxygen consumption between January and March and infer that experiments early in December might have given even lower values than those made in January.

Certain observations made in the southern states have indicated that the metabolism was somewhat lower during the warm weather. A series of measurements made on themselves in New Orleans by Hafkesbring and Collett (1924) were obtained for the most part at fairly low environmental temperatures. Thus, in the first three-quarters of the series with subject M. E. C., the external temperatures averaged well below 20°C., on several days being as low as 3° or 4°C. The subject had been lying on a couch for about thirty minutes before the metabolism determination was made, and breathed outdoor air during the experiment. The authors conclude that the basal metabolism is about 5 per cent higher in cold weather (3° to 10°C.) than in hot weather (20° to 27°C.). When one considers the extraordinary reaction shown by these two subjects to slight noises and other minor stimuli, one questions whether a study of such a subtle factor as the influence of season can be made suitably with such highly nervous individuals.

Another study made in New Orleans by Hafkesbring and Borgstrom (1926), although not dealing specifically with the metabolic influence of season, bears also upon this point, in so far as differences in climate may be considered to be typical of differences in season. Thus, they maintain

that the metabolism is from 14 to 18 per cent below normal in the warm environment of New Orleans. Corlette (1923) also found that the metabolism of people in the warmer climate of Australia was lower than that of people in the colder climate of New York, and McConnell, Yagloglou and Fulton (1924, 1925) state that the minimum metabolism occurs at temperatures of about 75° to 83°F.

This evidence might be considered as supporting the general view that during the cold weather more heat is produced to keep the body warm and the metabolism is therefore somewhat higher during the colder season. Since it is a prerequisite that prior to all basal metabolism experiments the subject should be comfortable and relaxed, it is a question as to how long a previous exposure to cold, even if moderate, would affect the metabolism. In a series of observations made by Gessler (1925) on himself on a good many days throughout one whole year, an extraordinarily close correlation between his basal metabolism and the outdoor temperature is noted. The metabolism was found to be highest in January, when the temperature was the lowest, and lowest in July when the temperature was the highest. Gessler's routine prior to the metabolism measurements called for a sojourn of about three to four hours in the hospital or laboratory at moderate temperatures, and then a minimum period of one-half hour during which he was lying, comfortably covered, on a bed. It would thus seem that the cold weather had affected his metabolism to such an extent that the effect was still apparent even after four hours' stay in a moderate temperature. Gessler's results are so striking that, if substantiated, they should call for the consideration in all further basal metabolism measurements of the time of year, and particularly the prevailing environmental temperature.

In 1927 preliminary announcement was made by Griffith and his colleagues of a long series of basal metabolism measurements on two normal men who were under observation for two years, and on three normal women studied for one year. These authors conclude that the oxygen consumption, hence the energy expenditure, is lowest in summer.

The possible significance of season has also been mentioned by Du Bois (1927) in his admirable book on basal metabolism in health and disease, in which he lists, among "additional factors affecting metabolism, the importance of which has been recently recognized or suggested," the "time of year."

Thus far in all basal metabolism experiments and in all summations of basal data, the possible effect of season has been almost entirely disregarded. The general conclusions of the earlier writers on the seasonal variability in metabolism are greatly at variance. But it is important to recognize at the outset that in studying this problem one has several factors to consider, primarily, perhaps, temperature, but certainly also the influence of sunlight (so strongly emphasized by Lindhard) and humid-

ity. Indeed, the various factors making up a tropical climate should ultimately be considered in such an analysis.

WELLESLEY COLLEGE STUDY ON THE INFLUENCE OF SEASON. To study specifically the influence of change in season upon the basal metabolism, the Nutrition Laboratory coöperated with the Department of Zoölogy and Physiology at Wellesley College in an extended series of observations upon young women students at this college. Obviously extreme tropical conditions were not met with at Wellesley, but there were the ordinary annual variations in sunlight and temperature, common to this latitude.

The subjects, twenty in number, were all undergraduates at Wellesley College and presumably in good health. They coöperated in every way, showed the keenest interest in the work and great seriousness of purpose. Realizing at the start that only those individuals who presented themselves for measurement at the appointed time, without fail, for a period of at least six months would be of any significant help in this study, they assumed entire responsibility for the series of observations. We wish to express our warmest appreciation of their coöperation.

Ideally, in order to secure a complete picture of the effect of season upon metabolism, the study should have included observations on each individual on two or preferably three consecutive days each month throughout an entire year. Practically, it was impossible for the young women who volunteered for this work to take too much time from their rather heavy programs of class work. It was believed, however, that if two consecutive periods of measurement could be obtained on one day each month with each girl for at least a year, the picture would be reasonably clear. In collecting the data the subjects were selected and the experimental program arranged for them without any regard for the menstrual period, in the initial belief that the metabolism would be but little, if at all, affected by menstruation. A certain number of menstrual days therefore inevitably appeared in the records and no effort was made to avoid them. On the other hand, no effort was made to include them.

The experiments were planned so that on each young woman two well-agreeing basal periods might be secured once each month throughout an entire year, save during the months of July, August and September, when the students were away from college. The general routine was as follows. The subjects, in pairs, slept at one of the Wellesley College laboratories the night preceding the basal metabolism experiments, in comfortable beds, under the same conditions under which they would normally sleep. Even in the winter the windows were open, and hence throughout the night they were subjected to the ordinary fluctuations in temperature due to the season. In the morning, after they were awake but before they got out of bed and before they had anything to eat, their metabolism was measured during two successive periods of about 10 minutes each. This same pro-

cedure was repeated practically once a month on each girl. The series of measurements are not complete for all the subjects, although records are available for all twenty for at least five different months, and there are groups of 5 and 8 subjects upon whom successive monthly observations (except for the summer vacation) were secured over periods of 15 and 13 months respectively. (See p. 54.)

The respiration apparatus used was the Nutrition Laboratory's most recent portable form (Benedict, 1925) in which, instead of valves, a small external rotary blower is employed. The carbon dioxide is absorbed in a bottle containing soda-lime and the oxygen consumption is represented by the contraction in the volume of air inside a spirometer. This spirometer is not placed in the direct air circuit but at one side, so that the air enters and leaves the spirometer in wave-like motions corresponding to each respiration of the subject, the main air current passing directly by the opening to the spirometer. Under these conditions a minimum amount of work is demanded from the lungs of the subject and there is no question with regard to the complete absorption of carbon dioxide or, more particularly, the degree of humidity in the air or gas inside the bell, so that the corrections for temperature and pressure are never in doubt. Two such apparatus were placed in the laboratory room, and two experiments were run at the same time. A Collins chronokymograph recorded graphically the excursions of the spirometer counterpoise pointer, and a time clock gave the time indications. The apparatus was continually inspected, kept in excellent order, and controlled for tightness in that a weight of about 70 grams was placed on the spirometer bell for the last five minutes of each test. If the general slope of the curve showing the oxygen consumption was materially altered during the time that the weight was on the bell, it was assumed that the apparatus was leaking and the experiment was discarded. Rarely did this occur.

It was expected that the first measurements would be somewhat high, owing to the novelty of the experience. To minimize this effect, the subjects were in every instance allowed to breathe through the apparatus for five minutes before their first experiment. They then lay for half an hour in complete muscular repose, when the first experimental period began, followed immediately by a second. The conditions with regard to environmental temperature, muscular repose and, for the most part, diet were ideal, for rarely was a high protein meal taken the evening before. But it was impossible to control the psychic state of these young women, and they undoubtedly were more or less influenced by the experiences typical in a college, such as possibly anxiety with regard to examinations and the anticipation of an approaching Christmas vacation.

The usual records with regard to age, body weight, height and pulse rate, the preliminary observation of the mouth temperature to prove the

absence of fever, and a short statement as to dietetic habits, hours of sleep, and activities the night preceding the test were obtained in each case. We wish to emphasize, however, that we believe pulse rates taken upon a subject breathing into a closed-circuit respiration apparatus may be liable to misinterpretation, owing to the distinct effect which the higher oxygen content of the air inside the closed circuit has as compared with the oxygen content of ordinary room air. When breathing high oxygen, one's

TABLE 1

Age, weight, height and average basal metabolism of young women used in study

SUBJECT NUMBER	AGE		BODY WEIGHT (WITHOUT CLOTHES)	HEIGHT	PULSE RATE	OXYGEN CONSUMED PER MINUTE	HEAT PRODUCED PER 24 HOURS		
							Total	Harris-Benedict prediction	Deviation of actual from predicted heat
	years	*months*	*kgm.*	*cm.*		*cc.*	*cal.*	*cal.*	*per cent*
I	18	6	61.6	174	68	192	1,334	1,477	−9.7
II	18	2	60.5	152	66	234	1,626	1,432	+13.5
III	22	1	50.4	165	62	169	1,174	1,339	−12.3
IV	21	1	46.0	163	53	183	1,271	1,298	−2.1
V	19	5	49.1	168	58	193	1,341	1,348	−0.5
VI	20	2	68.3	170	59	213	1,480	1,529	−3.2
VII	20	3	66.0	166	69	209	1,452	1,500	−3.2
VIII	19	10	59.8	165	63	194	1,348	1,439	−6.3
IX	19	10	55.7	165	72	244	1,695	1,400	+21.1
X	18	8	60.5	170	50	191	1,327	1,460	−9.1
XI	20	1	58.0	165	68	208	1,445	1,422	+1.6
XII	19	8	54.9	152	60	183	1,271	1,368	−7.1
XIII	21	11	70.9	170	69	211	1,466	1,545	−5.1
XIV	21	8	57.8	165	62	209	1,452	1,410	+3.0
XV	19	10	58.4	173	60	191	1,327	1,441	−7.9
XVI	18	9	62.0	158	62	191	1,327	1,452	−8.6
XVII	18	3	53.3	168	61	193	1,341	1,393	−3.7
XVIII	20	1	55.6	152	63	196	1,362	1,375	−0.9
XIX	21	2	66.2	165	62	211	1,466	1,495	−1.9
XX	20	3	50.3	160	57	204	1,417	1,339	+5.8
Average.....	20	0	58.3	164	62	201	1,396	1,423	−1.8

pulse rate may be lowered from 5 to 7 beats. In general, however, since the technique of enriching the air in the spirometer bell with oxygen was essentially the same throughout all the experiments, we believe that the pulse rates are comparable.

NORMALITY OF YOUNG WOMEN USED IN STUDY. In order to study the variability in the metabolism of these young women at different times of the year, it is first desirable to know what is their normal basal metabolism

and whether any of them have a metabolism markedly below or above normal standards. For this purpose we have recorded in table 1 the age and height of each subject and, since the changes in body weight throughout the period of experimentation were only slight, the average body weight. In addition, the average pulse rate and the average oxygen consumption, representing the average for all the observations made, are given, and finally, the total heat production (computed from the average oxygen consumption), the heat predicted from the Harris-Benedict formula for women (Harris and Benedict, 1919; Carpenter, 1924), and the percentage deviation of the actually measured from the predicted heat production.

As can be seen from these data, the twenty young women as a group are fairly homogeneous in age and physical characteristics. The actually measured metabolism and the predicted metabolism would therefore be expected to be in reasonably close agreement, particularly in so far as the group as a whole is concerned. The general experience in the Nutrition Laboratory and in a number of other laboratories, where women have been studied, is that groups of women tend to have a metabolism averaging about 5 per cent less than the predicted standards for women. Indeed, it has recently been definitely suggested that the standards for white women be lowered 5 per cent (Benedict, 1928b). It is of particular interest, therefore, to see whether the results with this group of twenty girls confirm this general thesis. Somewhat to our surprise, it was found that the metabolism of the group as a whole averages but 1.8 per cent below the prediction. That we had to deal with unusually healthy young women, who engaged considerably in athletic sports and general outdoor activities, may account for the fact that their metabolism on the average is a little higher than experience would have led us to expect. Indeed, with five of the subjects the deviations of the actual from the predicted heat production were plus, whereas ordinarily the values are definitely minus. This suggests that the subjects possessed unusual vigor.

There is no obvious reason for the unusually high metabolism of subject IX (+21.1 per cent) or of subject II (+13.5 per cent). These results, however, are quite in line with the experience at the Nutrition Laboratory and elsewhere, that even with as homogeneous a group as one comprised, for example, of ten presumably healthy medical students, at least one or more individuals will have a metabolism deviating more than ± 10 per cent from the standards. In the Wellesley College study those young women whose metabolism on the average for the year was high or, conversely, low, consistently had a high (or a low) metabolism at each monthly period of observation. The fact that with two of the subjects the measured heat production was more than 10 per cent above the normal standards is therefore not of significance in our study of seasonal effect.

The general conclusions to be arrived at from an inspection of table 1 are

that the young women used in this study were homogeneous in age and in physical configuration and had a normal metabolism in all but possibly two instances. Our study of the seasonal variation in metabolism is therefore not obscured by any pronounced abnormalities in these respects.

Since these young women may be adjudged to have a normal metabolism, these data supplement the list of normal basal metabolism data for women

TABLE 2

Variation in oxygen consumption of young women (post-absorptive) with change in season

(Values in cubic centimeters per minute)

SUBJECT NUMBER	OCTOBER (1926)	NOVEMBER	DECEMBER	JANUARY (1927)	FEBRUARY	MARCH	APRIL	MAY	JUNE	OCTOBER	NOVEMBER	DECEMBER	JANUARY (1928)
I	190	186*	192	188*	192*	205*	188	198	193*				
II	259	238	260	226	234	222	233	222	208				
III		184	163	172	170	173	171	154	164*	168			
IV	188	182	195	182	176	198	189	171	175	170			
V		191		185	203	196*	170	184	201		192	214*	
VI	201	226*		197	223	221	198	208*	234	215	208	232	189*
VII	223	202	213	196*	221		213	209*	211	212		202	199
VIII	209	199	196	188	202	183	184	199	188	180		192	202
IX		254		249	256	263	248	224	235	227	235	265	226
X		204	215	175		193	190	177	180	186	164	213	193†
XI	230	207	215*	230	208	227	217	197	185		187	199	194
XII		191	185	177	181	171	187	185*	186		190	184*	172
XIII	213	211*	214	211	208	211	209	219	220*	200	203		
XIV		199	212	217*	211	224	211	202*	217	205	205	200	
XV		200*	201	184	196	198	179	179*	214	179	177		
XVI		188	184	197	183	187	207	195	211	178	182	196	185
XVII		195	210	196	204*	203	192	185*	225	156*	174	184	193
XVIII	212	208	199*	183*	193	219	191	201	203	193	181	181	184*
XIX	227	240	223	199	206	212*	220	200*	221*	199*	191	209*	202
XX	205	205	223	193	196	217	207	214	203*	202	193	200	197

* Menstruating.

† Subject X had an oxygen consumption of 199 cc. in February, 1928.

published by Benedict (1928b), in which for the most part women of college age were purposely not included.

INFLUENCE OF SEASON UPON BASAL METABOLISM. The values for the oxygen consumption per minute, as noted with each subject for each month that she was studied, are given in table 2. Changes in body weight which occurred during the year were so small as not to justify computing the heat production per kilogram of body weight or per square meter of body surface,

and it is believed that the general picture will be well shown by these oxygen figures. The asterisks in the table indicate values determined on menstrual days. These results have not been excluded, since there is not sufficient uniformity in the data obtained on these days to indicate a definite effect of menstruation. In a few instances there is a hint, however, of a possible effect of menstruation, and further analysis of this factor in the metabolism of women will appear in a following paper.

Of the twenty subjects listed, no. IX has a metabolism which, according to table 1, is the highest above the prediction standard and, as can be seen from table 2, her metabolism remains high throughout the entire series of measurements. On the other hand, the metabolism of no. II, although high on the average according to table 1, shows a tendency to fall off during the time she was under observation. The metabolism of no. III, which averages 12 per cent below the prediction standard, remains reasonably uniform throughout the entire series of observations. It therefore seems clear that, although these particular subjects have either a very high or a relatively low metabolism, their results are perfectly suitable (except perhaps in the case of no. II) for use in a study of the seasonal change in metabolism. We have therefore excluded none of these subjects in our consideration of this question.

From table 2 the trend in the oxygen consumption for any individual subject may be followed. Owing to the irregularity in the number of subjects studied each month, averaging of the values for each month is not permissible. But an inspection of the data for individual subjects shows that in general the lower values occur during the winter. A striking exception is the second December observation, when five out of twelve subjects with whom the comparison may be made showed a pronounced increase in metabolism over the values obtained approximately two weeks before. The operator was under the impression that the anticipation of a departure from college for the 1927 Christmas vacation was the stimulus accountable for this particular rise noted with these subjects. The results are in no case uniform, but they represent fairly well the differences in metabolism which may be expected in measuring a group of young women from time to time. Certainly after the first few observations in the fall of 1926, however, the young women may be considered as more or less trained subjects. Hence we have no unusual psychic disturbances to deal with, except possibly the effect of anticipation prior to the 1927 Christmas vacation. This is not particularly noticeable in the 1926 December series except in two cases, although with eight other subjects the metabolism in the middle of December, 1926, was a little higher than it was in November, 1926, thus suggesting the possibility of a stimulus from an anticipation of the vacation. It is impossible in a study of this kind to rule out every factor save season in every case. The main characteristics of college

existence are undoubtedly more or less reflected in this group of young women, and it is possible that another group of individuals living an entirely different type of life might not show exactly the same results.

The variability in the values for individuals from month to month is such as to make general deductions perhaps open to considerable criticism, and in an analysis of the data as a whole certain features of the experiments should be carefully borne in mind. In the first place, it is highly probable, as already pointed out, that anticipation of the 1927 Christmas vacation may have acted as a stimulus to metabolism. In the second place, in the May observations seven of the twenty subjects were measured during menstruation, and subsequent special experiments on this problem have demonstrated a tendency for the metabolim to be low during the menstrual period. For this reason, as will be pointed out in a following paper, we believe that basal metabolism measurements on women should not be made on days when they are menstruating. There are likewise five instances in the June observations when the subjects were measured during menstruation. But it is to be noted that in May, contrary to June, the starred values representing the menstrual periods are usually lower than the values either for the month preceding or the month following, frequently noticeably so, and these low values result in lowering the general average for the month of May. In the third place, our technique was distinctly at fault in that we did not secure the average of two or three days' measurements on each young woman each month, but relied upon one single day's observations. Fortunately on this one day two experimental periods were, with but few exceptions, obtained in every instance. The variability in the metabolism of these individuals and particularly the varying stimuli occurring in any student's year at college are, however, such as to make reliance upon one day's findings open to serious criticism. This is strikingly brought out by comparison of the values in table 2 for any one subject from month to month. Thus, the difference between the lowest and the highest value in practically every instance amounts to from 15 to 25 per cent. Even with an especially well-trained, phlegmatic woman subject a difference of 14 per cent was found between the minimum and the maximum metabolism during a period of two months. Seemingly the metabolism of women is distinctly a labile process, but further consideration of the day to day variability in normal metabolism must be deferred until a later paper.

Bearing these features of the experiments in mind, we may proceed with an interpretation of the results, although stating frankly that they are by no means as decisive as we could wish.

The course of the metabolism throughout the year is somewhat difficult to follow from table 2, but may best be seen by plotting average values for each month. Unfortunately all the subjects were not studied every

month throughout an entire year. Five subjects (nos. XVI to XX) were, however, studied uninterruptedly for 15 months, or from November, 1926, through January, 1928, and eight (nos. XIII to XX) for 13 months, or from November, 1926, through November, 1927. Curves showing the course of the average oxygen consumption of these five and eight subjects, respectively, are given in figure 1. (See, also, table 3, p. 55).

It is common experience that complete uniformity in metabolism measurements from day to day with any one individual cannot be expected, and we have here in each of these curves a variability up and down which is quite in accordance with that ordinarily found with individuals. With a well-trained subject, leading an extraordinarily regular life and frequently measured in respiration experiments, a high degree of uniformity in metabolism from day to day has been found (Benedict, 1928a), but in ordinary life, particularly in student life of this type, there are likely to be considerable fluctuations in metabolism and strict uniformity in the measure-

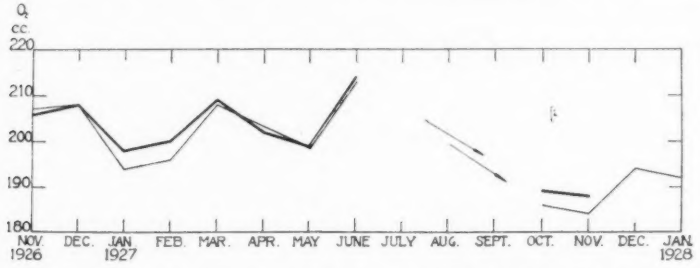

Fig. 1. Course of the oxygen consumption of young women with the change in season. The light black curve represents the average for five subjects, the heavy black curve that for eight subjects.

ments cannot be expected. On the other hand, if there is a general tendency for the metabolism to be higher in one period of the year than in another, it should be reflected in the curves for these groups of young women.

As can be seen from figure 1, the curves for the groups of five and eight subjects for the most part parallel each other reasonably well, so that one could consider only the curve for the group of eight, *i.e.*, the heavy black line. These curves show that there is a low metabolism during the winter, followed by a rise to a higher level in the spring. The interpretation of the curves during the spring, however, is complicated by the rather low level in May (possibly explained by the relatively large number of subjects measured in the menstrual period) and the high level in June. If one averaged the values for the months of March, April, May and June, the average would be perceptibly above the metabolic level for the two winter

seasons. That this is due to differences in temperature is by no means clear, and we are reminded of the suggestion of Lindhard (1910, 1912) that the metabolism may perhaps be altered by an increase of sunlight.

It is greatly to be regretted that measurements could not have been made during July, August and September for particular comparison with the second winter series, when a metabolism even lower than that during the first winter is noted. The higher level of metabolism in November and December, 1926, may or may not be interpreted on the grounds that the subjects were hardly sufficiently trained at that time to the experimental

TABLE 3

Variation in buccal temperature, pulse rate, and oxygen consumption of groups of young women with change in season

MONTH	MOUTH TEMPERATURE		PULSE RATE		O_2 PER MINUTE	
	5 in group	8 in group	5 in group	8 in group	5 in group	8 in group
	°F.	°F.			cc.	cc.
November, 1926	97.3	97.3	61	60	207	206
December, 1926	97.8	97.5	61	61	208	208
January, 1927	97.8	97.6	56	58	194	198
February, 1927	97.8	97.8	58	61	196	200
March, 1927	97.6	97.4	56	59	208	209
April, 1927	97.9	97.7	62	61	203	202
May, 1927	97.5	97.5	57	60	199	199
June, 1927	97.7	97.6	62	64	213	214
October, 1927	97.7	97.6	63	64	186	189
November, 1927	98.0	97.7	63	64	184	188
December, 1927	97.8		63		194	
January, 1928	97.9		62		192	

routine, for the observations made during these months represent only the second and third experiences of these subjects with the respiration apparatus. This again accentuates the error of not having measured the metabolism of each subject on at least two or three consecutive days each month, and although we believe this criticism does not affect our general conclusions, the betterment of technique in this respect is recommended in any future studies made to clarify completely the question of seasonal variation in metabolism.

Average monthly values for the oxygen consumption, the pulse rate, and the mouth temperature of the same groups of young women as are represented in figure 1 are given in table 3. The results for the oxygen consumption of course simply duplicate the picture shown by the curves. The variations in the pulse rate are, on the whole, small. The lowest pulse rates with both groups are, to be sure, found in January, more or

less in accordance with the low metabolism noted at this time. But there is certainly no evidence here of a marked relationship between pulse rate and metabolism. As already stated (see p. 49), pulse rates determined during respiration experiments when subjects are breathing oxygen-rich air are always liable to considerable error, inasmuch as the inhalation of this oxygen-rich mixture tends to lower the rate perceptibly. The general picture, however, of the pulse rates throughout the year is doubtless shown by these values with reasonable correctness, namely, that there is not a profound difference in the rate with the change in season, although the higher pulse rates appear to occur in June, October and November.

The mouth temperatures, as indicative of body temperatures, are helpful in considering the contention of Young (1920) of Australia, who finds a correlation between body temperature and metabolism. Although mouth temperatures are not so satisfactory as rectal temperatures, nevertheless, as can be seen from table 3, the mouth temperatures of our subjects during January and February do not differ significantly from those at other seasons of the year. Hence the lower metabolism in January and February cannot be ascribed to a low buccal temperature.

These findings are in contradistinction to those of Gessler (1925) and of Young (1920), but more nearly in conformity with the findings of Lindhard (1910, 1912). They do not support the contention of Kunde (1923) that the metabolism is lowest during July and August, and are contrary to the observations of Palmer (1914), whose metabolism was much higher in the winter than in the summer, a finding which is in accord with that of Hafkesbring and Collett (1924).

To plan and carry out a research of this kind involves operation on such a large scale that it is extremely difficult to duplicate the work. No one is any more aware than are we of the gaps in our data. In consideration of the fact that the differences in metabolism found with the changes in season are not strikingly large, these gaps are all the more to be deplored. Yet we feel justified in setting forth the thesis that the metabolism, at least of a group of college girls, tends to be somewhat lower in the winter months and higher in the spring and summer. This thesis is supported by the fall in metabolism noted between October and January with the control squad of Y. M. C. A. men studied by the Nutrition Laboratory at Springfield (Benedict, Miles, Roth and Smith, 1919).

This indication of a seasonal variation in metabolism should not be overlooked in any study of metabolism carried out over a long period. Thus, in considering the changes in basal metabolism caused by a superimposed factor such as a special dietetic regime, physical or athletic training, light, etc., one should not neglect a seasonal factor. It is clear from our study, however, that although the general trend of basal metabolism is strongly suggestive of an influence of season, further research regarding

this factor is imperatively needed. With the modern technique the greatest problem arising is not the degree of accuracy in the technique nor the skill of the operator, but the coöperation of the subjects. This cooperation, we believe, can only be secured in some college community, and the spirit of service and interest in scientific research now dominant in most of our college centers should make such group coöperation possible.

SUMMARY

With twenty young women, volunteer subjects at Wellesley College, the basal metabolism was measured once each month (with the exception of July, August and September) between October, 1926, and January, 1928, thus including two winter seasons. A respiration apparatus of the spirometer type was employed, and the oxygen consumption was graphically recorded. The average mouth temperature showed no significant fluctuations. The varying stimuli incidental to college life undoubtedly affected the individual monthly measurements, but the average values for the oxygen consumption strongly suggest that the metabolism tends to be at a low level in the winter and to rise to a higher level during the spring and summer. In many of the results there is a hint that the metabolism is lowered during the menstrual period.

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NORMAL MENSTRUATION AND GASEOUS METABOLISM

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The course of metabolism from day to day has been too little studied with normal individuals, although not infrequently determined with pathological patients. Daily observations on normal individuals mean a long, tedious accumulation of data which ordinarily show but small fluctuations in metabolism, and there is no particular stimulus to obtain such records because the results are not especially striking. A few hardy experimenters have carried out such tests, either on themselves or on willing, coöperative subjects. But in most instances normal basal metabolism measurements on any one subject have been few in number and made intermittently, not on consecutive days. With women these intermittent measurements have rarely included menstrual days, and the influence of menstruation can best be determined only if a long, uninterrupted series of measurements is available both on intermenstrual and on menstrual days.

In the literature regarding the metabolic studies on menstruation which have already been made, the conclusions vary widely. L. Zuntz (1924), whose interest in metabolism is of many years' standing, in his comprehensive article on metabolism and the sexuality of women, states that since there is a premenstrual increase in numerous functions, especially in the temperature, one is led to expect a rise in the respiratory exchange. The earlier experiments of Rabuteau (1870) and of Robin and Binet (1904) Zuntz claims are not significant on account of defects in their technique. He himself studied two women through numerous menstrual periods, in accordance with the Zuntz-Geppert method, and found that menstruation had no influence upon the metabolism. Salomon (1905) came to the same conclusion.

Gephart and Du Bois (1916) also found no difference in the metabolism of their artist's model, studied on the second day of the catamenia and four days later, and it is emphasized (Du Bois, 1927) that this model suffered no discomfort. Blunt and Dye (1921) record no definite change in heat production in a large number of observations on 14 women, the average values for the menstrual days being but 1.6 per cent lower than that for the other days. They conclude that their data give no indication of a ryth-

mical periodic variation in metabolism. Wiltshire (1921) found no change in the metabolism during menstruation. Sturgis (1923) reports results with a woman who had a very high metabolism due to a toxic goitre. Measurements made during six menstrual cycles showed that the metabolism was little, if at all, affected by the menstrual function. Lanz (1925) concludes that the basal metabolism is not influenced by menstruation in the case of normal healthy women.

Studying a group of young women with the main object of determining the effect upon metabolism of walking, Smith and Doolittle (1925) made a number of measurements while the subjects were standing, prior to exercise, but in the post-absorptive condition. The measurements were therefore not obtained under strictly basal conditions, which demand that the subject should be lying down, well covered. However, as a result of these observations the authors conclude that the menstrual period appears to be without definite influence on the standing metabolism.

In contradistinction to these findings are the records of Snell, Ford and Rowntree (1920). They note that a rather constant rise (averaging 10 per cent) in metabolism occurs during menstruation or in the premenstrual period, and that this rise is followed by a postmenstrual fall. They suggest that ovulation may be directly or indirectly responsible for this rise. The details of a special investigation carried out by Ford to determine the extent of the influence of menstruation they state "will appear shortly" in a separate communication, but we have been unable to find any reference to a later publication. Rowe and Eakin (1921) conclude that the menstrual cycle has a pronounced effect upon the metabolism, the highest point in the metabolic curve being in the premenstrual week. Wakeham (1923) also seems to have evidence that there is a premenstrual rise. He points out that the data of Blunt and Dye (who thought their results showed no connection between basal metabolism and the menstrual cycle) as well as his own work demonstrate that there is a distinct fall in metabolism during or immediately after menstruation. He also notes that there is a gradual return to normality within from 7 to 10 days after the menstrual period. But he lays greatest emphasis upon the premenstrual rise.

Hafkesbring and Collett (1924) rightly claim that many of the earlier observations are open to objection, inasmuch as the experimental periods were too short and at irregular intervals, but state that even in these earlier reports there is some evidence of a premenstrual rise and a menstrual drop in metabolism. From their own experiments they conclude that there is a definite tendency toward a rise before each menstrual period, a sharp drop on the first or second day of menstruation, unless interfered with by pain, and an intermenstrual minimum, the difference between the high and the low levels amounting to 5 per cent. Collett and Liljestrand (1924)

find that the oxygen consumption rises to a maximum 4 to 10 days after and again 15 to 19 days after the beginning of the menstrual period, reaches a minimum during the first or second day of the period and again 10 to 18 days later. They also note a premenstrual rise and a menstrual fall in the ventilation of the lungs (more or less in conformity with the conception of a cyclic rhythm in metabolism), but they state that the postmenstrual maximum and the intermenstrual minimum are not clear.

Kunde (1923) finds that there is no constant difference in the metabolism during the first four menstrual days as compared with that during the four days preceding the monthly period. But the tendency seems to be towards a lowering of the basal metabolism during the first four days of menstruation.

The literature on the influence of menstruation is summarized by Du Bois (1927), who comments that the menstrual effect is not yet clearly established in spite of all the experiments which have been made. Boothby and Sandiford (1924), in a brief review of the literature, state that they have observed not infrequently that pain or distress, whether of menstrual or other origin, will cause a rise in the metabolism.

Nutrition Laboratory experiments on influence of menstruation. It is clear from the evidence of the earlier writers that great differences of opinion obtain regarding the influence of menstruation upon metabolism. This is, as some writers have pointed out, in large part due to the fact that many of the observations were intermittent and not sufficiently consecutive, that normal individuals (not "hospital normals") were not readily found who would subject themselves to the tedious routine of a long series of consecutive metabolism measurements, and that with those who have been studied over rather long periods obvious pain or discomfort attended menstruation. We feel, therefore, as if Boothby and Sandiford (1924) have emphasized the most important factor to be taken into consideration in studying the influence of menstruation, that is, that one must rule out the question of pain and discomfort before an intelligent study of this normal function can be made.

With exactly this point in mind we took advantage of the extraordinarily placid nature and normal functioning of a young woman (Miss W.) to study the basal metabolism during menstruation, uncomplicated by discomfort or feeling of malaise. This woman, a professional artist's model, has, during the last twelve years, served intermittently as the subject of respiration experiments in the Nutrition Laboratory, and during this time she has never had to postpone an appointment for illness of any kind. She has found that her menstrual periods are normal in flow and in time, without pain or distress, and that they do not interfere in the slightest with her posing. Indeed, most of our earlier work with her was done under abnormal conditions involving exposure to cold, even during the menstrual

period, and only rarely were measurements made when she was clothed and under ordinary basal conditions. It would thus seem as though she were an ideal subject for this particular study.

In collecting our data on the normal basal metabolism of this woman, with special reference to the effect of age (Benedict, 1928a) we found that she had been studied on 32 menstrual days under the usual prescribed basal conditions. On these days the metabolism frequently, although by no means invariably, was so far below her average metabolism on intermenstrual days as to challenge attention. It therefore seemed necessary, in studying the effect of age with her, not to include measurements made on any menstrual days. The normal data available on this basis show that the metabolism of this woman has remained singularly constant over a period of nearly twelve years. Thus, on 89 intermenstrual days during these twelve years her average metabolism was as follows: oxygen consumption per minute 189 cc., total heat production per 24 hours 1316 calories, heat production per kilogram of body weight per 24 hours 22 calories, and heat production per square meter of body surface per 24 hours 810 calories. Almost invariably her metabolism was lower than the Harris-Benedict prediction, on the average -4.5 per cent. This average deviation is what we commonly expect with women, for the present-day predictions for women are believed to be 5 per cent too high. Indeed, a lowering of these predictions has recently been recommended (Benedict, 1928b).

The individual percentage deviations[1] of the actual from the predicted metabolism on each of the 89 intermenstrual days have been published in a preceding paper (Benedict, 1928a). Analysis of these data shows that plus deviations are found on only nine days and of these only two are greater than $+1.5$ per cent. The majority of the values are minus and yet only seven are greater than -10 per cent.

The percentage deviations[1] on 32 menstrual days, between December 4, 1920, and January 18, 1928, are given in table 1. They are in all but three cases minus. On April 22 and November 23, 1921, and on January 13, 1922, they are $+2.9$, $+2.3$, and $+0.5$ per cent, respectively. The average deviation for the entire series of 32 days is -7.5 per cent, or 3 per cent lower than the average of -4.5 per cent noted for the intermenstrual days. Fourteen of the 32 values are -10 per cent or more, averaging -12 per cent. This proportion of large minus values is greater than that noted in the intermenstrual series and points toward a distinctly lower metabolism of this woman during the menstrual period. But the fact that on the menstrual days a few plus values were found and frequently values arithmetically higher than the average intermenstrual deviation of -4.5

[1] In all cases these percentage values are based upon measurements derived from at least two experimental periods, usually three or more, and not infrequently five.

per cent shows that menstruation did not invariably produce a depression in metabolism, so far as this evidence is concerned.

In a coöperative study between the Nutrition Laboratory and Miss Florence L. Gustafson, of the Department of Zoölogy and Physiology at Wellesley College, the basal metabolism of a group of 20 young women was measured approximately once each month for a period of over a year, with the special purpose of determining the effect of season on metabolism. In this series of measurements no particular emphasis was laid upon making the experiments on intermenstrual or menstrual days, but the convenience

TABLE 1

Deviation of measured from predicted (Harris-Benedict) basal metabolism of Miss W on 32 menstrual days

DATE	DEVIATION	DATE	DEVIATION
1920	*per cent*	*1922*	*per cent*
December 4	−14.7	January 13	+0.5
December 6	−16.2	January 14	−4.5
December 7	−13.7	January 16	−10.0
December 29	−1.6	January 17	−12.0
1921		February 11	−8.0
January 21	−11.1	February 13	−12.0
January 22	−8.6	February 14	−11.2
January 24	−9.2	February 15	−10.2
January 25	−4.3	March 10	−11.1
January 26	−6.3	March 11	−9.6
February 23	−5.5	*1924*	
Feburary 24	−3.5	January 25	−0.6
March 26	−10.0	*1927*	
April 22	+2.9	April 8	−6.5
November 21	−1.4	*1928*	
November 22	−11.6	January 17	−11.0
November 23	+2.3	January 18	−12.5
December 16	−4.6		
December 21	−4.1		

of the program for the young women was the first consideration. As a result of this study not a small number of observations were made on menstrual days, each based upon two well-agreeing periods. In the tabular presentation of this seasonal material, Gustafson and Benedict (1928) indicated with asterisks the measurements made during the menstrual periods. Of their twenty subjects all but six were measured at least once on a menstrual day, and in two instances (subjects I and XIX) on five different menstrual days. From an inspection of their table one can only agree with their finding that, although there is a hint that the metabolism is lowered, the results are so irregular as to preclude any definite

conclusion as to the influence of menstruation upon metabolism. Occasionally, strikingly low points are observed on the menstrual days, notably with their subjects XIV, XVII and XVIII. The picture is by no means clear, for not infrequently a high metabolism is noted upon these days, especially with their subjects I, V, VI, XIII and XIX. It is thus evident that more than one day's measurement is essential in order to establish the influence of menstruation, and that the result for an individual day may be affected by so many variable factors as to obscure the issue. This is quite in line with the conclusion of Blunt and Dye (1921).

It is highly probable that the life of these college women was far less uniform than the life of our subject, Miss W., and hence extraneous factors might easily have played a larger rôle in the college series. At this point, however, we believe it necessary to emphasize that in any study of superimposed factors, such as that of season, the better technique (in lieu of the evidence brought forth in this paper) would call for a ruling out, with women, of all measurements made on menstrual days. This is one of the most important features of our study, although it does not alter in the slightest the general conclusions drawn by Gustafson and Benedict as to the effect of season, for in that case a large group of individuals was studied and the irregularity in menstrual values is such as not to affect the general picture of the influence of season.

In view of the suggestion of a depressed metabolism during menstruation noted in the accumulation of normal basal data for our subject, Miss W., and partly supported by the Wellesley College data, it was planned to measure Miss W.'s basal metabolism, in so far as possible, on consecutive days through three menstrual periods. In such a study it is obvious that the ordinary fluctuations obtaining from day to day between the menstrual periods must be taken into consideration in interpreting any findings on menstrual days. That is, one cannot assume a uniform metabolism from day to day, even without the intervening factor of menstruation, for although of unusually placid temperament and leading a very regular life, this woman, as well as everyone else, is subjected to the fluctuations in psychic stimulus and physical activity incidental to any normal life. It seemed, however, as if a study of this kind over a period of two months or more should give a fairly clear picture of the metabolism during the menstrual periods, although it was realized that only eight or nine menstrual days at the most could be expected in a two months' study.

In this new series with Miss W. the most modern technique in the Nutrition Laboratory was employed. The respiration apparatus was of the closed-circuit type, with an external blower, an external soda-lime bottle, and a spirometer not connected directly in series. It has already been described in detail (Benedict, 1925). This apparatus, as are all apparatus in the Nutrition Laboratory, was carefully controlled in two

ways. In the first place, the metabolism of this subject (which has been found to be extraordinarily regular from period to period on any one day) as measured on this type of apparatus was compared with that measured on the same day with other types of respiration apparatus. Close agreement was found in the values thus obtained. The apparatus was further tested by means of an alcohol check test. A known weight of alcohol was burned in the apparatus, the oxygen required to burn this definite amount was calculated, and the result compared with the amount of oxygen measured by the apparatus. The comparison was most satisfactory.

The experiments proper with Miss W. began on February 8, 1928, and continued daily, with few interruptions except on Sundays, until April 13. On the first four days only two experimental periods were run each day, but thereafter the metabolism measurements were usually based upon from three to six periods. These results were further checked by basal measurements made with another type of apparatus which was being tested at that time. We therefore have perfect confidence in the values for the oxygen consumption of this woman on each individual day. The subject was always post-absorptive, and the absence of a febrile temperature was noted prior to each experiment. Although the experiments were always made at the laboratory temperature of about 20°C., with the subject comfortably covered and in complete muscular repose, the possible effect upon the metabolism of the previous exposure to the outdoor temperature was considered, and a daily record of the outdoor temperature was kept.

The results obtained in this latest series of measurements with Miss W. are recorded in table 2. The outdoor temperature during the two months' study ranged from −6.5°C. to +18.9°C., but no correlation between the external temperature and the metabolism is to be found. The pulse rate remained singularly uniform, ranging from a maximum of 64 beats on March 6 to a minimum of 48 on April 6. Of greatest significance is the variability in the oxygen consumption per minute. In an earlier paper, in which the normal intermenstrual metabolism of this subject was reported for a period of twelve years (Benedict, 1928a), attention was called to her rather uniform metabolism from day to day, although it was pointed out that the uniformity was not such as to justify the assumption of a definite base-line for any continued study. The values obtained in the 1928 series show the fluctuations which may normally be expected even with a woman of unusually placid temperament and in excellent health, both during the complete menstrual period and the intermenstrual period. As can be seen from table 2, the lowest oxygen consumption between February 8 and April 13 was 169 cc. on February 16, a menstrual day. The highest value was 193 cc. on February 25, and the next highest was 189 cc. on March 21, both intermenstrual days.

The oxygen consumption is also recorded graphically in figure 1, the menstrual days being indicated by the letter M. Aside from the general irregularity appearing from day to day, the curve for the oxygen consumption is low during the menstrual periods, especially during the first two periods, that is, from February 14 to February 17 and from March 10 to

TABLE 2

Basal metabolism of Miss W during three menstrual cycles

DATE (1928)	NUMBER OF PERIODS	OUTDOOR TEMPERATURE	MENSTRUATING	PULSE RATE	OXYGEN CONSUMED PER MINUTE	DATE (1928)	NUMBER OF PERIODS	OUTDOOR TEMPERATURE	MENSTRUATING	PULSE RATE	OXYGEN CONSUMED PER MINUTE
		°C.			cc.			°C.			cc.
February 8	2		No	60	174	March 7	5	2.0	No.	62	180
February 9	2		No	59	179	March 8	2	1.5	No	58	187
February 10	2		No	52	170	March 10	4	−1.2	Yes[2]	55	178
February 11	2		No	56	175	March 12	3	2.2	Yes	50	172
February 13	5		No	56	177	March 13	2		Yes	51	171
February 14	3		Yes[1]	53	172	March 14	3	8.9	Yes	57	172
February 15	4	9.0	Yes	55	172	March 15	3	4.4	No	54	174
February 16	4	6.3	Yes	51	169	March 16	3	0.6	No	54	173
February 17	5		Yes	55	171	March 17	3	1.7	No	56	179
February 18	4		No	54	174	March 19	3	2.2	No	54	182
February 20	4		No	55	178	March 21	3	3.9	No	50	189
February 21	4	−6.5	No	55	183	March 23	3	0.6	No	49	185
February 23	4	8.7	No	56	178	March 28	6	1.1	No	52	175
February 24	4	7.0	No	63	185	March 29	4	1.7	No	62	183
February 25	4		No	55	193	March 30	4	3.9	No	57	178
February 27	5	−1.0	No	51	185	April 3	5	6.7	No	55	180
February 28	4	−5.0	No	56	183	April 4	4	12.2	Yes	54	179
February 29	4	2.4	No	55	176	April 5	4	13.3	Yes	49	180
March 2	6	3.5	No	61	181	April 6	4	18.9	Yes	48	176
March 3	5	−1.3	No	54	182	April 10	8	2.2	No[3]	51	178
March 5	6	−2.0	No	58	183	April 12	4	1.7	No	60	177
March 6	6	−6.2	No	64	183	April 13	4	10.6	No	54	175

[1] Began February 13 at 5 p.m.

[2] Began early in morning.

[3] Stopped April 8 in morning.

March 14. In the third period, from April 4 to April 6, the metabolism, although not high, is not, relatively speaking, as low as in the other two periods. Another salient feature of the chart is the tendency for the curve to reach a high point about in the middle of the intermenstrual periods. This occurs both in February and in March. This high point so far precedes the menstrual period, however, as to make it impossible to consider

this as the so-called "premenstrual rise." It is better characterized, perhaps, as an intermenstrual rise. The situation is somewhat obscured by the fact that following the menstrual period in April there is no immediate tendency for this rise to assert itself. The experiments should have been carried somewhat further into April, but it became necessary to terminate the series.

The pulse rates have been plotted on the same chart with the oxygen consumption, and it is more than a coincidence that the curve for the pulse rate during the menstrual days is for the most part low, in conformity with the low oxygen consumption. On the other hand the high pulse rates do not occur upon the days of the highest metabolism.

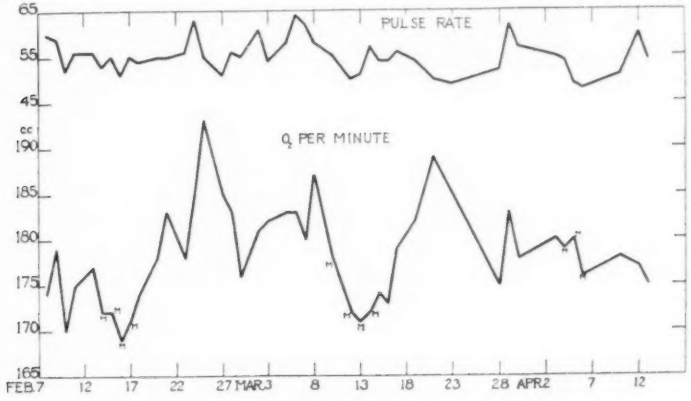

Fig. 1. Oxygen consumption (cc.) and pulse rate per minute of Miss W. from day to day throughout a period of two months, including three menstrual cycles. The menstrual days are indicated on the oxygen curve by the letter M.

We believe our evidence indicates that with a normal woman, whose menstruation is physiologically normal, there is a distinct tendency for the lowest metabolism to occur during the menstrual days and for a high metabolism to appear about a week after the last day of menstruation. This low metabolism during menstruation, followed by the rise, constitutes a variability in the basal metabolism of a normal, healthy woman which is relatively large. Thus, the difference between the minimum oxygen consumption of 169 cc. and the maximum of 193 cc. in the month of February is 24 cc., or 14 per cent, considering the lowest metabolism as the basis. Similarly in March, the rise from 171 to 189 cc., that is, a rise of 18 cc., is an increase of 10 per cent. The variability in the metabolism of Miss W. on 89 intermenstrual days has already been discussed in a previous paper (Benedict, 1928a). Although the measurements on these days and on 32

menstrual days during the same period of time were not sufficiently consecutive to make a strict comparison of the two groups of data possible, in general one finds that the variability in the metabolism on the 89 intermenstrual days alone is not so great as that noted in the entire series of measurements including both the 89 intermenstrual and the 32 menstrual days. It would thus appear as if menstruation is a real factor lowering the metabolism, and that during the intermenstrual period there is a rather marked increase, followed by a few days of relative uniformity in the metabolism.

In a previous paper (Benedict, 1928a) we stated that we believed it was desirable to avoid menstrual days in studying the normal metabolism of women. But as can be seen from table 2, even on the intermenstrual days, such as from February 18 through March 8, there is a wide variability in the metabolism. The same holds true between March 15 and April 3. On the other hand, there are periods as, for example, between March 2 and March 7, when the metabolism remains remarkably uniform for five days. Again from March 28 to April 3 the deviation is only from 175 to 183 cc. Perhaps the greatest uniformity for a few consecutive days is secured during the actual menstrual period. In any event it is important in studying the metabolism of women, to record in what phase of the menstrual cycle they are measured. It is our plan to make further experiments with Miss W., although it is difficult to maintain a long series of uninterrupted observations to study this problem alone, and we can only hope to secure further evidence in connection with other extensive series of metabolism measurements made for entirely different purposes. We believe, however, that we have here with a normal woman, whose monthly function is unaccompanied by pain or discomfort and hence is most physiological, the clearest evidence that the basal metabolism is low during the menstrual days.

SUMMARY

Intermittent measurements of the basal metabolism of a normal woman (Miss W.) over a period of 12 years furnish the data for the comparison of the metabolism on intermenstrual and menstrual days. The deviation of the measured from the predicted (Harris-Benedict) heat production averaged −4.5 per cent for 89 intermenstrual days and −7.5 per cent for 32 menstrual days. The difference of 3 per cent suggests that menstruation may lower the metabolism, and this is further emphasized by the preponderance of deviations greater than −10 per cent, which occurred on the menstrual days.

With a group of Wellesley College students, whose basal metabolism was measured once each month for an entire year, primarily to determine the influence of season, occasional observations were also secured on

menstrual days. Although there was a wide variability in the results obtained on these days, there was a hint in some instances of a depression in metabolism during menstruation.

To study the influence of this factor in greater detail, an extensive series of practically consecutive daily measurements, extending over two months and including three menstrual cycles, was made with the same subject mentioned above (Miss W.). Because of her unusually placid temperament and good health and because she experienced no pain or discomfort as a result of this normal physiological function, it is believed that, in so far as possible, the menstrual factor alone was involved in this study.

The measurements made included the pulse rate and the oxygen consumption, the latter being recorded graphically by means of a closed-circuit respiration apparatus connecting indirectly with a spirometer.

The outdoor temperature seemed to have no correlation with the metabolism. The pulse rate was usually lowest when the oxygen consumption was low, but the high pulse rates did not occur at the same time as the high oxygen consumption. The oxygen consumption in general was lowest and most uniform during the menstrual period, and highest about one week after menstruation ceased. Menstruation would thus appear to be a real factor lowering the metabolism.

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HEMORRHAGE HYPERGLUCEMIA

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Ten animals which had been subjected to various experimental abuses were, at the end of each experiment, killed by exsanguination. All the animals were under deep amytal anesthesia. In seven of the ten animals it was found that the last portion of blood removed was richer in reducing substance than the first portion, though between these two portions the time was short and the flow of blood continuous. These experiments do suggest that the amount of blood drawn from the animal per kilogram of body weight may influence the concentration of the reducing substance in the sample.

E. L. Scott (1914) discusses a series of experiments which suggests that the concentration of reducing substance in the whole sample varies inversely with the amount of blood withdrawn *at any time*, and is independent of the actual amount of blood drawn. To explain this phenomenon, he utilizes the fact already established, that during hemorrhage there is a leaching of the tissue fluids from the tissue spaces into the blood vessels. This explanation seems plausible, but is based on the assumption that the capillaries can take up fluid from the tissue spaces in such a way as to exclude the glucose which that fluid contains, an assumption for which no evidence exists, and which is very difficult to allow. Recently, G. A. Clarke (1928), in listing factors which may play a part in a sudden increase in the rate of fall of glucose concentration of the blood following pituitrin administration, mentions "dilution of the blood," but objects to this explanation on the grounds already mentioned.

EXPERIMENTAL. The experiments here reported were performed on eighteen healthy cats under deep amytal anesthesia. After a preliminary starvation of 24 hours, they were given intraperitoneally 60 to 90 mgm. of amytal in 10 per cent solution per kilogram of body weight. Apparently the animals, for the most part, did not object to the injection. Anesthesia was complete in from 10 to 30 minutes, and there was very little preanesthesia excitement. When anesthesia was complete, the animals were put upon a warm operating board, tied, and an artery, usually the right carotid, was isolated. From 1 to 5 hours later, the prepared artery was

carefully cannulized, the occluding serrefine removed, and the blood collected in beakers as fast as it flowed from the cannula. Between the "beaker samples" were collected "sugar samples" of about 2 cc., and the blood kept fluid by means of sodium oxalate crystals. No animal ever struggled or had asphyxial convulsions. The respiration became deeper and faster, then gradually shallower, and stopped in from 5 minutes to 1½ hours after the bleeding. Exsanguination required from 4 to 5 minutes. No blood was collected after an arbitrary five-minute period, in order to avoid the last few drops of markedly asphyxiated blood. Reducing substance was determined by the method of Shaeffer and Hartman (1920) with the aid of the modified table of Dugan and Scott (1926), and hemoglobin concentration was estimated according to the method of Cohen and Smith (1919), the first sample collected being put at 100 per cent.

TABLE 1

Control. Cat I, M. Weight 3.6 kgm. 2-11-28	Blood drawn, cc. per kgm.	0	6	10	16	20	25	29	31
	Glucose, per cent	0.083	0.089	0.085	0.090	0.088	0.101	0.156	0.243
	Hemoglobin, per cent.	*100*	*100*	*96*	*91*	*87*	*84*	*82*	
Control. Cat XV, M. Weight 4.2 kgm. 3-30-28	Blood drawn, cc. per kgm.	0	8	14	18	25	32		
	Glucose, per cent	0.108	0.106	0.117	0.114	0.117	0.129		
	Hemoglobin, per cent.	*100*	*97*	*93*	*85*	*88*	*85*		
Adrenalectomy. Cat V, M. Weight 3.3 kgm. 2-24-28	Blood drawn, cc. per kgm.	0	7	12	17	21	27	30	
	Glucose, per cent	0.076	0.078	0.078	0.080	0.077	0.082	0.140	
	Hemoglobin, per cent.	*100*	*97*	*95*	*91*	*95*	*107*	*97*	
Adrenalectomy. Cat III, M. Weight 3.3 kgm. 2-21-28	Blood drawn, cc. per kgm.	0	8	15	19	23	29	34	
	Glucose, per cent	0.197	0.197	0.197	0.203	0.203	0.210	0.215	
	Hemoglobin, per cent.	*100*	*94*	*91*	*88*	*88*	*85*	*82*	

This procedure was carried out on eight animals, which constitute the control series. In a second series, the ten animals were subjected to double adrenalectomy, but were otherwise treated as were the controls. A variable time was allowed to elapse between the operation and the exsanguination, in order to determine the effect of various levels of glucemia upon the "hemorrhage sugar curve."

Results. The results obtained without and with adrenalectomy were so much alike that they will be discussed together. In the appended table are shown the results of several experiments from each series. These show that acute hemorrhage is accompanied by an increase of reducing substance in the outflowing blood. The increase is slow and often insignificant at first, but more rapid toward the end, giving high values for the last sample. In order to reduce the time necessary for complete exsan-

guination, two animals were decapitated. The blood-sugar was affected in much the same way as in the more slowly-bled animals.

Special attention is called to the hemoglobin figures: expressed in percentages of the initial value, they invariably show a decrease in the hemoglobin concentration of the blood. This is interpreted as due to the following factors:

a. The early drop in hemoglobin is due to the quick fall in blood pressure, and expresses a valiant attempt on the part of the organism to maintain an adequate circulation by dilution of the blood.

b. Once after the blood-sugar has risen, factor *a* is reinforced by the rise in osmotic pressure of the blood, tending to draw tissue fluid into the blood and so further to dilute the blood.[1]

Studied individually, some of the curves obtained in this series are flatter than those of the controls. Taken collectively, however, the flattening becomes insignificant. If the blood-sugar percentages are plotted against the amount of blood drawn per kilogram of body weight, it is found that the curve thus obtained has a positive slope. The same is true if the sugar concentration of the mixed samples is thus plotted.

Discussion. How is the rise in blood sugar concentration accompanying hemorrhage to be explained? Despite the marked dilution of the blood, as evidenced by the hemoglobin concentration curves, the blood-sugar concentration remains constant, or is actually increased. The rise may be due to one or more of several factors:

a. The sugar content of the diluting fluid may be as great as, or even greater than that of the blood itself. *b.* Glucose mobilization may occur incident to the fall in blood pressure and to marked asphyxia. This is supported by the fact that the greatest rises in blood sugar occur late in the hemorrhage. *c.* A combination of both these factors seems the probable explanation.

That the diluting fluid can be compared to distilled water, as is often done, is contrary to reason. Lymph, which presumably resembles the diluting fluid under conditions here imposed, is variously reported to contain from 0.08 to 0.1 per cent of reducing substance—as much as the blood. The experiments here reported support this claim. We found that we could withdraw from the animals from 8 to 30 cc. of blood per kilogram of cat before any rise in blood-sugar occurred. This gives an average of 16 cc. of blood per kilogram!

The relatively late occurrence of hyperglucemia suggests that this may be due to asphyxia, with its resulting effects upon the central nervous system and the tissues in general.

[1] Author's unpublished experiments.

SUMMARY

1. Experimental evidence is presented which shows that cats under amytal anesthesia, when exsanguinated from an artery, yield blood increasingly rich in reducing substance. A marked increase in glucose occurs after an average of 16 cc. of blood per kilogram of animal has been removed (8 to 30 cc. per kilogram).

2. This is not due to an asphyxial outpouring of epinephrin by the adrenal glands, because removal of both adrenals has no influence on the shape of the curves.

3. As evidenced by the fall in hemoglobin, hemorrhage has a marked diluting effect on the blood.

4. The "diluting fluid" poured into the blood during hemorrhage contains at least as much glucose as the blood.

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PHYSIOLOGY OF THE CORPUS LUTEUM

I. The Effect of Very Early Ablation of the Corpus Luteum upon Embryos and Uterus

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In 1903 and 1910 L. Fraenkel published a series of experiments which indicated that the corpus luteum of pregnancy has an important function in connection with implantation and maintenance of the embryos. His experiments were performed upon rabbits, in which the embryos become attached to the uterus about the 8th day after mating. After removal of both ovaries at any time during the first six days, the uterus was always found empty when examined ten or more days later. If the corpora lutea of pregnancy were excised or destroyed by cautery the same effect was produced, while control operations (involving resection or cauterization of ovarian tissue with survival of some of the corpora lutea) were followed by pregnancy in about half the cases.

Fraenkel's original series of 1903 was small, and its publication evoked so many criticisms that a much larger number of experiments were reported in 1910. There are obvious faults in the experiments; for example, in some of the cases in which pregnancy did not occur it is not demonstrated that the animals had actually ovulated (cf. cases xxxiv, xlii, xliv). However, there can be no reasonable doubt that on the whole Fraenkel's control experiments interfered with pregnancy far less frequently than those in which all the corpora lutea were removed.

In 1907 Leo Loeb showed that during the first days following ovulation the uterus is in a special physiological condition by which placentation is facilitated. Using the guinea pig for his experiments, Loeb exposed the uterus on the seventh day following an unfertilized ovulation (at which time the embryos, had they been present, would have been about to attach themselves) and placed within the uterine cavity a small foreign body such as a piece of glass. Within four days the site of such a trauma was marked by growth of a tumor of the endometrium composed of decidual tissue. In its simplest form this beautiful experiment could be performed by simply scratching the endometrium at the proper time of the cycle. Loeb found that he could not produce deciduomata at other times of the

cycle than the first week after ovulation, and that removal of the ovaries or cauterization of the young corpora lutea also prevented the development of the artificial deciduomata.

In 1910 Bouin and Ancel began the work of placing a morphological foundation under Fraenkel's results by demonstrating the occurrence of actual changes in the rabbit uterus concomitantly with the development of the corpora lutea. It will be remembered that in the rabbit there is no spontaneous ovulation, and hence no formation of corpora lutea except after coitus. Bouin and Ancel permitted rabbits to mate with males sterilized by vasectomy, and thus secured the development of corpora lutea without pregnancy. Under these conditions the uterus was found to become enlarged and hyperaemic; its epithelium, both superficial and glandular, underwent mitotic proliferation, and the crypts and glands increased their complexity of ramification until there was produced an elaborately specialized condition of the endometrium (see figs. 2, 3, 5) which Bouin and Ancel interpreted as indicating a preparation for implantation. These changes were found to reach their maximum about the eighth day after ovulation, and were followed by retrogressive changes from about the tenth day onward, until at the twenty-fifth day the uterus had returned to the resting condition. Ancel and Bouin have also at various times (1910, 1924) reported that removal of the corpora lutea by excision or cauterization causes failure of the process of endometrial proliferation.

The experimental results which have been quoted have been confirmed a number of times, and have been supported by a mass of histological evidence from various species (see Corner, 1923, for references) indicating that in all mammals so far studied a state of proliferation of the endometrium accompanies formation and maturity of the corpus luteum. The so-called "premenstrual" stage of the human endometrium is presumably of the same nature.

The conclusion that this post-ovulational change in the uterus is functionally necessary to implantation is, of course, a *post-hoc* assumption, however plausible. Apparently no one has attempted to observe what actually happens to the ova in consequence of experimental procedures such as those suggested by Fraenkel, Bouin and Ancel, and Loeb.

The following experiments were planned to test the results of Fraenkel and of Ancel and Bouin, and to determine the actual fate of the ova following very early removal of the corpora lutea.

The rabbit was chosen as subject of the experiments for the same reasons which no doubt led to its choice by previous workers, namely, the anatomical suitability of its long, fusiform ovary for surgical experimentation, and also the special nature of its reproductive behaviour. Female rabbits do not exhibit periodic oestrus, but when kept under favorable conditions will accept the male at almost any time. During the hours following mating

a group of follicles begin to mature. Rupture of these follicles and entrance of the ova into the Fallopian tubes occurs about ten hours after mating. The first segmentation occurs about the end of the first day; by the end of the third day the embryos are in the blastocyst stage, about 0.1 mm. in diameter (excluding the zona pellucida). At about the 80th hour the embryos enter the uterus. At the end of the fourth day they measure about 0.3 mm. in diameter. From the fourth to the seventh day the blastocysts float unattached in the uterine cavity. Implantation occurs on the eighth day.

As the preliminary stage of each experiment a doe was mated, usually to two bucks in succession, but sometimes to one only. Insemination was proven by demonstration of spermatozoa in vaginal smears. The female was thereupon again isolated until conclusion of the experiment. Operations were performed 14 to 20 hours after mating, under ether anesthesia, with sterile precautions. In some experiments the ovaries were exposed by means of a single median abdominal incision, in others by two flank incisions.

In the first group of experiments (table 1) bilateral oöphorectomy was performed 14 to 18 hours after mating, when the ova had been in the tubes 4 to 8 hours. Six such cases are listed, together with a seventh (rabbit 38) in which it was intended to leave part of the ovary intact, but the remnant was destroyed by necrosis due to impaired circulation, thus resulting in loss of all ovarian tissue. The animals were autopsied at various times from $4\frac{1}{2}$ days to $7\frac{3}{4}$ days after mating. At autopsy the entire reproductive tract was removed, the uterine cornua washed out with normal saline solution by insertion of a hypodermic needle, and the tissues prepared for histological study.

As shown by table 1, the embryos were found in the uterus in each case; they were all in the early blastocyst stage, and were degenerate, as shown by opacity and wrinkling of the blastocyst wall. Figure 7 shows the litter

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Fig. 1. Section of uterus of rabbit 30, showing unproliferated endometrium after ablation of corpora lutea. × 7.

Fig. 2. Section of uterus of rabbit 11, showing proliferation of endometrium in animal having part of one corpus luteum. × 7.

Fig. 3. Section of uterus of rabbit 5 (control experiment), 8th day of pregnancy, section taken between implantation sites. Typical progestational proliferation. × 7.

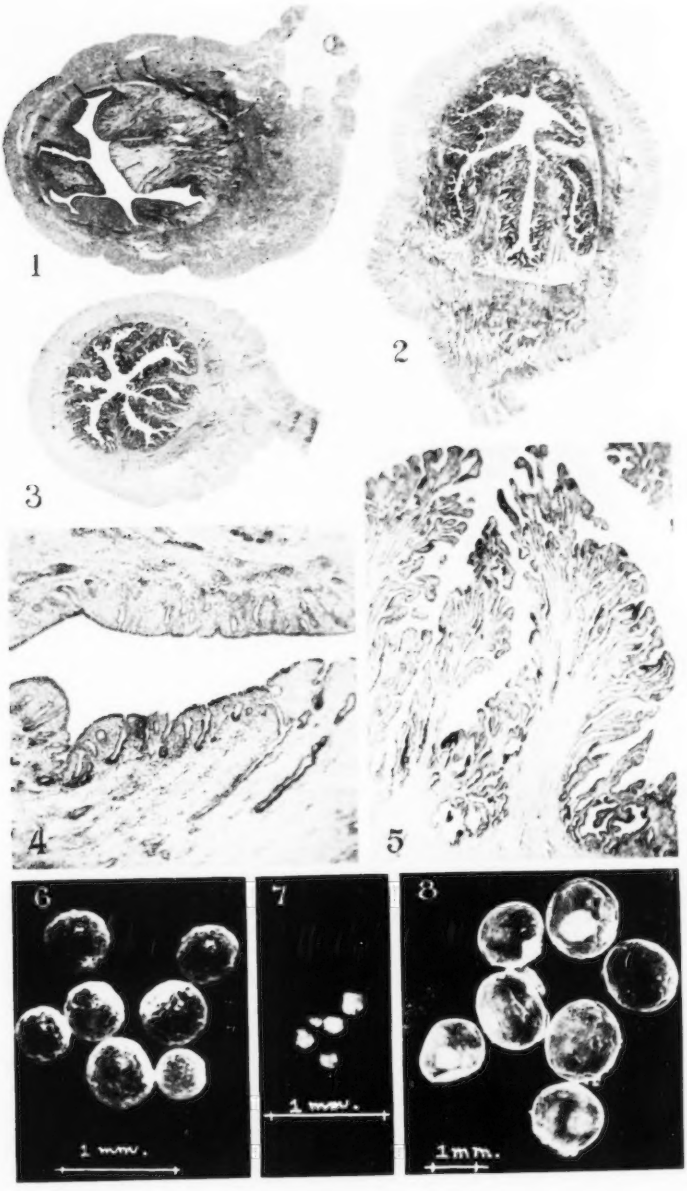
Fig. 4. Enlarged view of portion of endometrium of rabbit 30 (cf. fig. 1). × 40.

Fig. 5. Enlarged view of portion of endometrium of rabbit 5 (cf. fig. 3). × 40.

Fig. 6. Normal embryos of rabbit 33 (control experiment), 5th day of pregnancy. × 17.

Fig. 7. Degenerate blastocysts of rabbit 34, 5th day of pregnancy. × 17.

Fig. 8. Normal embryos of rabbit 37 (control experiment), 6th day of pregnancy. × 6.5.


1
2
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found in rabbit 34, in contrast with the normal embryos found in rabbit 33 (fig. 6), a control animal (see table 2). The embryos pictured in figure 7 are typical of those found in all the does of group I. When these dead blastocysts were measured, it was found that their diameters measured from 0.2 to 0.4 mm.; normal embryos of the rabbit of the 5th day after mating measure about 0.5 mm., and on the 8th day 4.0 mm. It is evident that death of the blastocysts occurred soon after they reached the uterus.

GROUP I. BOTH OVARIES REMOVED AT 14-18 HOURS			
No.	Autopsied	State of Embryos	Prolif
1 *	4 1/2 d.	degenerated 0.2 mm. diam.	0
18	4 1/2 d.	" 0.2 " "	0
34	4 3/4 d.	" 0.15-0.2 " "	0
3	5 1/2 d.	" 0.2 " "	0
2	7 1/2 d.	" 0.4 " "	0
4	7 3/4 d.	" 0.3 " "	0
36	5 3/4 d.	" 0.45 " "	0

GROUP II. CONTROL OPERATIONS AT 17-18 1/2 HRS				
No.	Op.	Autopsied	State of Embryos	Prolif
33		4 3/4 d.	7 Normal 0.5 mm.	+
24		4 3/4 d.	7 Normal 0.6 mm. 4 degen.	+
27		5 3/4 d.	1 Abnormal 1 mm.	+
37		5 3/4 d.	7 Normal 2 mm.	+
28		6 3/4 d.	3 Normal, shield stage	+
5		7 3/4 d.	5 Normal, 8-10 somites	+
21		8 3/4 d.	1 Normal somite stage	+

GROUP III. ALL CORPORA LUTEA EXCISED AT 15-20 HRS.				
No.	Op.	Autopsied	State of Embryos	Prolif
16		4 1/2 d.	4 Early degen. 0.4 mm.	0
19		4 3/4 d.	4 Unseg. ova in tubes	0
30		5 3/4 d.	No. emb. (ovulation +)	0
31		5 3/4 d.	" "	0
32		5 3/4 d.	" "	0
10		7 1/2 d.	4 deg. blastocysts 0.2 mm. in tube	0

Tables 1 to 3

Sections taken through the middle of the uterine horn showed that no progestational proliferation had taken place in these animals. The endometrium was in the typical resting state, and in some of the experiments even showed traces of castrate atrophy, having a smooth surface with few crypts and no glandular proliferation.

Figures 1 and 4 illustrate clearly the state of the uterus as found in animals of this group and group III.

Summary. Following bilateral oöphorectomy 14 to 18 hours after mating (4 to 8 hours after ovulation) the ova develop to the early blastocyst stage and are transported to the uterus; the endometrium does not undergo the proliferation normally characteristic of early pregnancy, and the blastocysts die soon after entering the uterine cavity.

In the second group of experiments (table 2) operations were done at 17 to 18 hours after mating. In 6 cases one ovary was removed and the other ovary was cut through or half-resected; in a seventh experiment (rabbit 21) one ovary was divided and the other was half-resected. Ruptured follicles were seen in each case, and one or more were present in the intact portion of the ovaries. The long narrow shape of the rabbit's ovary, and its blood-supply by two or three distinct branches of the ovarian artery, permit such resections to be made without danger to the surviving ovarian tissue. The small diagrams in the second column of table 2 indicate by solid black areas the portion of the ovarian tissue left intact in each experiment. From one-fourth to three-fourths of the total ovarian mass was thus removed.

The rabbits were autopsied at $4\frac{3}{4}$ to $8\frac{3}{4}$ days after mating. In each case the presence of normal ovarian tissue containing corpora lutea was confirmed by sectioning the surviving portion of the ovary.

In all of these experiments embryos were found in the uterine cavity, and in all but one (rabbit 27) they were normal in appearance and of the size found in normal pregnancy at the corresponding time after mating. Figures 6 and 8 illustrate normal embryos of rabbits 33 and 37, obtained on the 5th and 6th days after mating, respectively. In the two killed at the time of implantation or later (rabbits 5 and 21) the blastocysts were normally implanting. In one animal (rabbit 27) only one embryo could be recovered, and this was degenerate. The endometrium of all the animals of this group had undergone typical progestational proliferation, as illustrated by figures 3 and 5.

Summary. After removal of one ovary and transection or half-resection of the other, and after similar procedures involving damage to both ovaries and loss of one-fourth to three-fourths of the ovarian tissue, with survival of one or more of the corpora lutea, the embryos are transported into the uterus and develop normally, and the endometrium undergoes typical progestational proliferation.

In the third group of experiments (table 3) operations were done at 15 to 20 hours after mating. For these operations rabbits were selected which proved at the time of abdominal exploration to have all the ruptured follicles grouped at one end of the ovary so that they might all be excised without removing all the ovarian tissue. From the small diagrams in table 3 it will be seen that in two of the rabbits one-half the ovarian tissue was

allowed to survive; in two more, one-fourth of the ovarian tissue survived; and in the fifth animal (rabbit 16) about one-sixth survived. In rabbit 10 one ovary was removed and the corpora lutea in the other ovary were destroyed by a fine galvanocautery. The complete removal of all the corpora lutea of these animals was confirmed by serial section of all the remaining ovarian tissue at the time of autopsy.

The rabbits were autopsied at $4\frac{1}{2}$ to $7\frac{1}{2}$ days after mating. It will be seen from the table that the fate of the embryos was not the same in all six cases. In rabbit 16 four degenerating blastocysts were found, measuring about 0.4 mm. in diameter. In rabbit 19 no embryos were found in the uterus, but there were two unsegmented ova in each Fallopian tube. In rabbits 30, 31 and 32 no embryos or ova could be found either in uterus or tubes, although the occurrence of ovulation in these three cases had been proven by the finding of typical freshly ruptured follicles in serial sections of the portions of the ovaries removed at the operation. It has already been mentioned that the occurrence of insemination was checked in every animal by the finding of spermatozoa in vaginal smears taken immediately after mating. In rabbit 10, 7 degenerating blastocysts, the largest 0.2 mm. in diameter, were found in the uterus. In all of these cases progestational proliferation had not occurred, the endometrium presenting the typical resting state as found at the time of mating (figs. 1, 4).

It is not easy to understand why the fate of the ova was so different, after resections and cauterizations of the ovaries, from that of the ova in the animals which were deprived of both ovaries. The discrepancy can hardly be ascribed to differences in the anatomical condition of the Fallopian tubes, since in the two groups the number of cases in which one or both tubes were involved in adhesions was about the same. One cannot be as certain that physiological conditions were alike in the two groups. It is undoubtedly more difficult to do a neat resection than a complete oöphorectomy, and the trauma to the tubes and mesosalpinx is greater in the former operation. Moreover, the partially resected ovary is exposed to continued traumata in the form of ligatures, haematomata and adhesions actually involving ovarian tissue.

The most likely conjecture, therefore, is that disordered conditions arising in the ovary, and affecting the transportational function of the Fallopian tube, led in these experiments to delay of movement of the ova and even to their loss.

Summary. After removal of all the corpora lutea (recently ruptured follicles), with survival of one-sixth to one-half of the ovarian tissue, the endometrium does not undergo the proliferation normally characteristic of early pregnancy. In such experiments the ova do not develop after the first three days, but their exact fate is subject to certain obscure conditions probably arising from the experimental trauma.

Joublot has recently (1927) repeated the experiments of Bouin and Ancel with variations designed to reveal what amount of corpus luteum tissue is necessary to bring about proliferation of the endometrium. In ten experiments rabbits were mated to vasectomized bucks. At laparotomy 31 hours after mating, some of the corpora lutea were destroyed with the cautery, leaving others to carry on their function. It was found that two corpora lutea were sufficient to elicit the proliferation completely; one corpus luteum was not sufficient for full development of proliferation, and a part only of one corpus luteum was insufficient.

Two of the present experiments bear upon the question. In rabbit 27 of table 2, only one corpus luteum was present in the surviving ovarian tissue; in this animal, autopsied $5\frac{3}{4}$ days after mating, typical proliferation of the endometrium occurred, but only one embryo was present, and it was abnormal. In rabbit 11, not listed in the tables, an effort was made to destroy all the corpora lutea by the cautery, but the serial sections showed that about one-half of one corpus survived the procedure. In this animal the endometrium had undergone proliferation, as shown in figure 2. These two experiments agree in general with Joublot's results, differing only in that in rabbit 11 a smaller quantity of corpus luteum tissue sufficed to induce proliferation than Joublot thinks necessary.

CONCLUSIONS

The foregoing experiments confirm the existence of progestational proliferation of the rabbit's uterus during the earliest days of pregnancy, and support the discovery of Bouin and Ancel that this effect is dependent upon the corpora lutea. They also confirm Fraenkel's view that the corpora lutea are necessary to implantation of the embryos; and they suggest that normal implantation depends upon the progestational proliferation of the endometrium. They also indicate that the uterine proliferation is necessary not only for implantation, but for the nutrition or protection of the free blastocysts during the period of three or four days between arrival in the uterus and implantation.

Finally, these results point strongly to the existence of a special action of the corpus luteum upon the endometrium, bringing about progestational proliferation.

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THE MECHANISM OF INTESTINAL PERISTALSIS

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The experiments which are reported in this paper have been spread over several years and I have been assisted by the following members of my staff to whose efforts I will have occasion to refer: W. G. Mackersie, G. C. Currie and M. I. Sparks.

Before describing the experiments it seems advisable to discuss the terms which will be employed in describing the movements observed, owing to the confusion now prevailing in the literature.

a. *The rhythmic or pendular movement.* A contraction followed quickly by relaxation of the smooth muscle. Both phases of approximately equal length. The work of Gasser (1926), following on that of Gunn and Underhill (1914) and Alvarez and Mahoney (1922), has proved that they are myogenic, or rather that they do not depend on the plexus of Meisner or Auerbach. Whether the intramuscular plexus described by Tiegs (1925) is concerned in their production is not known. We will consider then the movements as myogenic. Circular and longitudinal coats are said to contract in harmony with each other in any given area (Bayliss and Starling, 1899; R. Magnus, 1905). Some observers (Schneller, 1925) consider that they are progressive, i.e., for the circular coat, the contraction of one area being succeeded by that of the next aboral area. Cannon (1901) appears to consider that they do not progress.

As Thomas and Kuntz (1926b) have shown, these rhythmic contractions are not abolished by nicotine in large doses equivalent to 2 grams per kilogram or 1:500 in the water bath. In this respect they resemble the so-called peristaltic waves in the pyloric portion of the stomach and the antiperistaltic waves of the large gut which according to Thomas and Kuntz (1926b) also continued after large doses of nicotine. I have long been of the impression that these waves in the large intestine and in the pyloric portion of the stomach resembled either the tonus waves sometimes seen in the small gut or else the rhythmic waves, rather than true peristaltic waves. I feel pretty sure of this position as regards the antiperistaltic waves of the large gut in guinea pigs, which have in the haustra the frequency of rhythmic movements rather than that of peristalsis.

b. *The peristaltic movement.* The original definition of Bayliss and Star-

ling (1899) was a ring of contraction progressing aborally preceded by an area of inhibition due to a coördinated reflex carried out by a local mechanism which included the plexus of Auerbach. On page 110 they speak of inhibition and relaxation (cessation of movement and fall of tonus?). In their tracing on page 107 there appears to have been a cessation of the circular but not of the longitudinal rhythmic movements below the peristaltic ring. For the rabbit and guinea pig, evidence has been accumulating that there is no cessation of rhythmic activity (Alvarez and Zimmermann, 1927; Baur, 1925) preceding the peristaltic wave. Further evidence is presented in this paper. Nor is there any evidence of a preceding area of decreased tonus. Further, Bayliss and Starling (1899) picture the peristaltic wave as a narrow band or ring From my observations on guinea pig and rabbit I am led to agree with the description given by Von Oettingen, Sollmann and Ishikawa (1928). And I will employ the word to mean the following: a shortening of the circular and longitudinal coat in a given area producing a ring which gradually broadens aborally so that a more or less considerable length is constricted, the whole then relaxing rapidly from the oral end. There is no inhibition of the rhythmic movements preceding the wave nor any fall of tonus. In the contracted area the rhythmic movements may possibly be absent and they are certainly reduced owing to the great shortening of the coats. I agree with Trendelenburg (1917) that the two coats are out of phase with each other, the longitudinal preceding the circular by about half a wave length or less. Baur (1925) has described certain experiments, particularly with colocynth, in which this relationship does not prevail, but I have not observed such a change in normal circumstances and am not as yet convinced that this occurs.

c. *Tonus.* A state of length on which rhythmic movements are or may be superimposed. The amount of tonus can be judged best by the rapidity of elongation to increasing tension, the lower the tonus the more rapid the elongation, provided of course that the elongation is produced so slowly as not to produce active responses or may be seen before the active response occurs. Tonus may be more marked in one coat than in the other. The amount of tonus may be judged perhaps by the use of a relatively large amount (1:1,000,000) of adrenalin. The greater the elongation the higher the preëxisting tonus.

d. *Tonus waves.* Slow changes in length increasing to a maximum with a decline. Their period may be long or short or the amount great or little. It may be noted that what are apparently tonus waves occurred in some of Gasser's (1926) preparations of ganglia free strips (e.g., as in his fig. 1).

Methods. The Trendelenburg method employed has been described previously (Henderson, 1923). The rate of increase of pressure has always been 10 mm. water pressure per 15 seconds. The bath solution has been

that of Fleisch (1922). Care has been taken to keep the aeration ample and steady. In other experiments a method has been employed which was devised independently but which in essentials so closely corresponds with that of Baur (1923) that it is referred to as the Baur method. It has been found that the most active strips of gut were obtained when the animals were given urethane and then an intraspinal anesthesia with novocaine. In rabbit gut the peristaltic waves with the optimum pressure develop slowly and are less frequent than with guinea-pig gut. It was found advantageous to use relatively young rabbits, though adult animals have also been employed. Only a few of the numerous experiments performed can be referred to in detail by means of protocols or tracings.

EXPERIMENTAL: *Section A. 1.* Henderson (1923) described the phenomena which occurred in a Trendelenburg gut which he described as peristaltic fatigue, namely, that peristaltic waves occur in groups. He pointed out that when this group formation was occurring the groups appeared at roughly regular intervals of time. This has also been observed by Baur (1923) and by Sollmann and is a normal phenomenon. Henderson further pointed out that a pinch applied between groups to the oral end of a strip of gut always caused a local contraction but only occasionally and especially late in the interval was a peristaltic wave produced, and then a single wave and not a series. On electrical stimulation with an alternating current may be produced also either local contraction or a single peristaltic wave. This is seen in a well marked form in tracing figure 4 (Henderson 1923). Grouping has been seen in rabbit gut and in this animal it is evident that rhythmic movements are present even when peristaltic ones are not and in spite of a maintained internal pressure.

A. 2. Another phenomenon which has been frequently observed but is especially well marked when high magnification of the longitudinal movements is used is well shown in figure 1. Interposed roughly midway between two peristaltic groups (longitudinal record alone is shown) there is a peculiar shortening with an increased evidence of the very slight rhythmic movements characteristic of guinea-pig gut, but no peristaltic group. This

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Fig. 1. Guinea-pig gut. Longitudinal movements only are shown with high magnification.

Fig. 2. Rabbit gut. A. Showing longitudinal movements, shortening of longitudinal coat on raising pressure. Increase of longitudinal after decreasing pressure. Effect of pilocarpine.

B. After 0.2 per cent nicotine. Irregular small longitudinal waves. On raising pressure tonus waves. Levers immediately above each other at lowest points of waves.

Fig. 3. Guinea-pig gut. Modified method described in text. 2.12.23.

Fig. 4. Guinea-pig gut. Modified method described in text. 2.1.24.

Fig. 5. Rabbit gut. Modified Trendelenburg method described in text. 2.5.26.

Fig. 6. Diagrammatic drawing of nerve connections in gut.

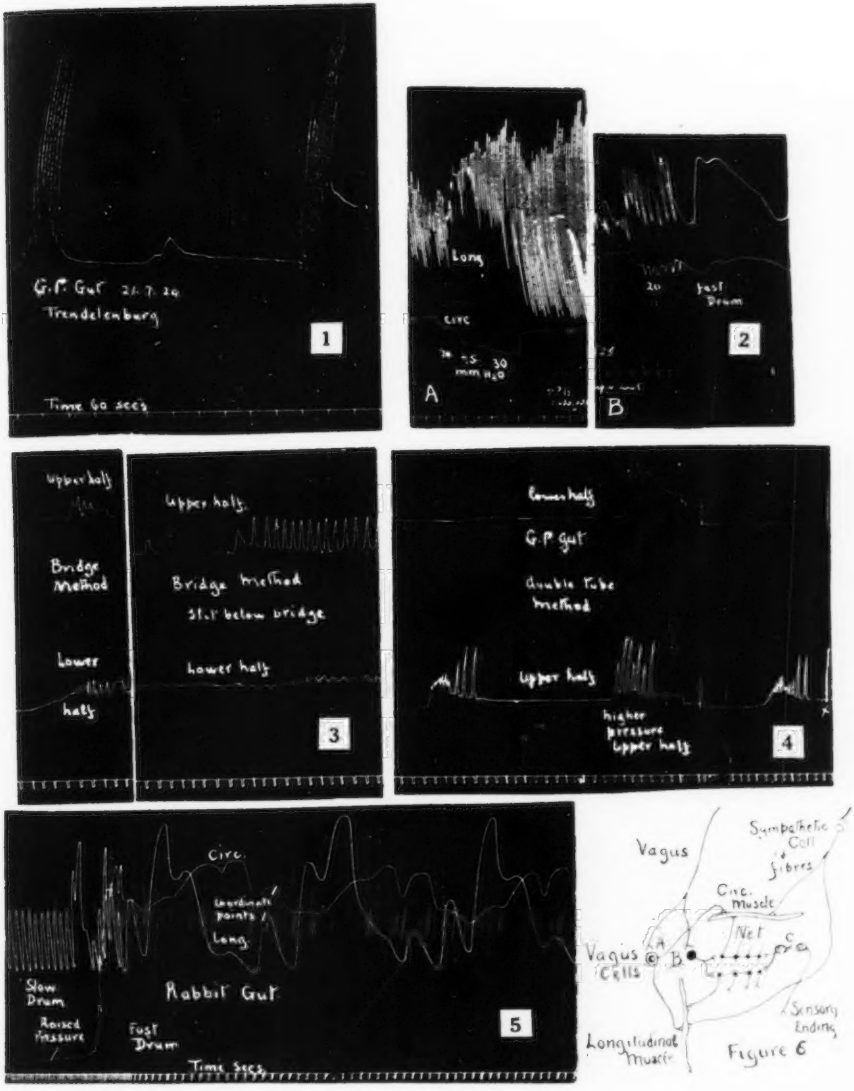
G.P. Gut 21.7.20
Trendelenburg
1
Time 60 secs
Long
Circ
A
2
Fast Drum
B
Upper half
Bridge Method
Lower half
Upper half.
Bridge Method
Slit below bridge
Lower half
3
Common half
G.P. Gut
Double Tube Method
Upper half
higher pressure Upper half
4
Circ.
Coordinate points
Long.
Slow Drum
Raised Pressure
Rabbit Gut
Fast Drum
Time Secs
5
Vagus
Sympathetic Cell
Fibres
Circ. Muscle
Vagus Cells
Net
Sensory Endings
Longitudinal Muscle

Figure 6

has been frequently seen and in many experiments performed with Baur's method where long strips are used and where grouping frequently occurs, a shortening of the longitudinal coat and an increase in rhythmic activity is frequently a sign that a peristaltic group or wave is about to occur. This appears even more strikingly in rabbit gut. But in some instances, and especially when the gut has been used for some time, this activity fades away without peristalsis, as occurred in the tracing. This tracing, however, shows further that a peristaltic group may occur without the preliminary shortening (second group in tracing). This increase of rhythmic movements preceding a peristaltic series has also been noted by Von Oettingen, Sollmann and Ishikawa (1928).

A. 3. A guinea-pig gut (M. I. S.) immersed in Fleisch solution and placed in the ice box at a temperature of 2°C when placed in Trendelenburg's apparatus will show peristalsis and rhythmic movements at the end of 6 hours: rhythmic longitudinal movements and weak peristalsis (26.11.27) at longer periods. No peristalsis was found after longer intervals than 12 hours, save in one case after 18 hours where weak movements possibly peristaltic were seen after treatment with hypertonic saline. In all other cases no peristaltic response was obtained after 16 hours, even with prolonged warming, though the rhythmic movements were active at much longer periods. In a further series of cases in which rabbit gut was used (V. E. H.) no peristaltic response was obtained after 6 to 8 hours even with prolonged warming and when the longitudinal rhythmic movements were ample. It was noted, however, that the preliminary phase of shortening which precedes peristalsis and which is often very marked with rabbit gut occurred. However, it was quite evident that rhythmic movements persisted when peristaltic movements were in abeyance.

B. 1. Nicotine. It was reported (Henderson, 1923), that peristalsis did not occur after the addition of nicotine in a strength of 1:600,000 to the nutrient bath. In experiments in 1925 with rabbit gut it was noted that though peristalsis could not be obtained after the addition of nicotine in the above strength, addition of larger doses tended to cause more marked rhythmic movements of the circular coats, especially when the gut was distended though peristalsis did not occur. The appearance of the paper of Thomas and Kuntz (1926a) has led to the repetition of some of these experiments and to using much higher concentrations.

It has been shown by Thomas and Kuntz (1926b) in dogs that rhythmic movements persist even after large doses of nicotine. An attempt was made to reduplicate these experiments in the rabbit. It was found that large doses of nicotine such as used by them led in rabbits to a rapid fall of blood pressure and even death. However, even after doses of 2 grams per kilogram, the gut showed active rhythmic movements in the animal, though the blood pressure was very low, almost zero, and the heart barely

beating. This gut activity may have been due to asphyxia owing to the low pressure, though artificial respiration was used. Strips of gut were then removed and set up in the Trendelenburg apparatus. Rhythmic movements appeared rapidly but no peristalsis was produced by any pressure. The preliminary shortening reaction, however, occurred. Chloroform was added to the bath until the rhythmic movements ceased. The effect of raising the internal pressure was tried from time to time and as long as rhythmic movements were evident the preliminary shortening occurred. When they ceased, however, no shortening but rather a lengthening of the gut occurred when it was distended.

B. 2. Pieces of rabbit gut or guinea pig in good tonus were set in Trendelenburg's apparatus and after peristalsis had been obtained by increasing the internal pressure and the pressure had been lowered again, nicotine hydrochloride in solution of the pH of the bath was added to produce a strength of 1:1,000 or 1:500. In all cases there was a very marked shortening of the longitudinal coat with prompt relaxation to a lower tonus than previously. Circular tonus, however, increased greatly, the gut becoming small and round when the internal pressure was zero. Longitudinal rhythmic movements were of less amplitude as a rule while circular ones were more evident. In some cases tonus waves of the longitudinal coats of short duration occurred which in guinea pig gut were often larger than any previous rhythmic movements, were but slightly slower, and showed some irregularities which appeared to be due to small rhythmic waves. In the higher concentrations the same increase in circular tonus occurred, longitudinal movements of all kinds were less in amplitude than before, circular more evident. On raising the pressure the gut was less distensible but in no case did peristalsis occur even with high pressures. The shortening reaction of the longitudinal coat persisted. In several cases in rabbit gut rapid circular movements which involved considerable areas 2 to 3 cm. of the total length of 8 to 10 cm. occurred. These were of a much more rapid rate than the peristaltic wave and approximated that of rhythmic waves. In other cases slow tonus-like waves occurred in the circular and also apparently in the longitudinal coat, which resembled peristalsis but on a faster drum both contractions, longitudinal and circular, appeared to occur at the same time. This phenomenon was also observed in strips which showed low circular tonus when taken, as for example in the following protocol.

5.5.28. Strip of ileum 6 cm. long. Rabbit had not responded well to urethane. Ether was given but when the abdominal cavity was opened made marked struggling movements leading to displacement of viscera and much handling. Strip showed very good longitudinal rhythmic movements, great shortening and increase of movements with internal pressure of 20 and 25 and 30 and gut distended readily and quickly, figure 2, A. Little tonus response to pilocarpine 1:400,000, little fall of tonus

with adrenalin but cessation of movements. Nicotine hydrochloride 1:500 caused a temporary shortening of longitudinal coat with prompt relaxation and subsequent irregular but small longitudinal waves. Gut less distensible to increase in pressure to 10, some longitudinal shortening. Rested: pressure to 20, little circular distention but irregular longitudinal and circular movements, figure 2, B. An area 2.5 cm. long, its oral end 3 cm. from the aboral cannula, contracts circularly and longitudinally, and a separate area 2 to 4 cm. from the oral end and not reaching the first, contracted while the first relaxed slightly. Then the oral 2 cm. and the intervening area shortened and contracted, so that the whole gut was short and almost emptied, then relaxation which was general. Occasionally the final contraction seemed to progress. Higher pressures led to cessation of movement and gradual dilatation.

An analysis on a faster drum showed that the expulsion curve and the longitudinal curve were synchronous in time relations. Such secondary waves on the curve as occurred were also synchronous. Watching the gut, and on the curve, the main rise appeared to be due to a non-progressive contraction of two areas, succeeded by a more general contraction. Owing to absence of good rhythmic waves it was difficult to estimate whether the large waves were peristaltic or not. They were more flattened at the apex than peristaltic waves usually are. It will be noted that in figure 2, B (Thomas and Kuntz, 1926b) the large waves have superimposed small waves and may well be marked tonus waves of the same type.

Conclusions, *Sections A and B.* The facts adduced in the above paragraphs may be summed as follows. As Trendelenburg has shown, an increase of internal pressure leads to peristaltic activity. The waves may occur in a long series or in a series of groups. When grouping occurs the cessation between groups is not consequent on an inability of the muscle fibres to contract as they respond to mechanical and electrical stimulation and rhythmic movements persist even when peristaltic ones are temporarily absent. Iced gut fails to show peristalsis at a time when rhythmic movements are present or even increased. Nicotine in relatively low or in high concentrations prevents the production of peristaltic waves, though rhythmic movements are active. From the premises it seems fair to conclude that peristalsis requires the adequate functioning of some nerve mechanism and probably one containing ganglionic relays which rhythmic movements do not.

Fleisch and Wyss (1923) described some experiments by which they considered they had proved the existence of neurogenic conduction in the intestinal wall. The method was in brief to take a section of guinea-pig gut about 4 cm. in length attaching its upper end directly to a writing lever while the lower end was attached to a short lower lever the other end of this lever being connected to a second writing lever. The strip was placed vertically in an oxygenated water bath of Fleisch solution. Midway the gut was grasped by its margins by clamps some 7 mm. wide one on each side. The clamps could be adjusted and fixed so that they stretched a

bridge of gut tightly between them. Now on stretching or pinching one end of the strip, not only the proximal piece of gut responded by an active movement but also the end beyond the bridge responded with a similar movement. This experiment has been repeated many times by myself and by W. G. M. and G. C. C., though with a slightly different technique. The difference lay in the fact that the gut was placed horizontally in the bath, each of the attaching threads running over a pulley to its lever. Or in experiments with G. C. C. the ends of the gut were attached to right angle levers. In no case where the bridge of gut between the clamps was tightly stretched have we been able to get conduction from one end to the other. A closer examination of Fleisch's tracings shows that the movements in response to stimulation were exceedingly small. Measurements of the tracings and considering the magnification of the lever, shows that the gut, on either side of the bridge can have shortened from an initial length of 2 cm. only about 3 mm. which is very small indeed. With G. C. C. some further experiments were performed with gut from small rabbits, in order that we might be able to judge of the activity of the preparation by the presence of rhythmic movements, which are frequently very small and inactive in guinea-pig gut. Both ends showed good rhythmic movements. The stimulated, usually pinched end, showed a temporary shortening or increase in size of the movements but there was no transmission to the other end.

The only explanation that we can offer of the differences between our results and that of Fleisch was that the stretching of the bridge was such as to interfere with nervous conduction, or that Fleisch had muscular conduction across the bridge owing to his not having stretched the bridge tightly enough. This appears the more probable as with a looser bridge we did get conduction but were able to see movements in the part of the gut constituting the bridge. Fleisch states that conduction was abolished in his experiments by atropine $1:10^8$ and cocaine $1:10^4$ and that the failure of conduction could be removed by washing. Having failed in repeating the fundamental experiment we have not tried the action of these drugs.

The method of Fleisch discussed above seemed applicable to the study of conduction of a nervous impulse set up by peristalsis and led to the following experiments.

Section C. 1. A section of guinea-pig gut some 10 to 12 cm. long was fixed by two laterally applied clamps which were adjusted so that the gut was stretched tightly to form a bridge. Fluid was then allowed to enter the oral end through a cannula until peristaltic movements were set up. The inlet cannula was small, light, and bent at right angles, and was supplied with fluid by a very light flexible rubber tube which descended vertically to the cannula from a mechanically moved aspirator bottle, as described by Henderson (1923). The longitudinal movements of peristalsis

alone were recorded by a thread attached to the cannula and passing round a pulley to a lever. The other end was attached to a similar lever. At first the aboral end was not provided with a cannula and the part of the gut below the bridge was empty. In this case pressure never rose in the lower section even if fluid passed the bridge and no waves of a peristaltic character appeared in the lower half. The aboral end was then provided with a cannula and pressure bottle and the pressure raised in the lower half but not sufficiently to cause peristalsis. Even then peristaltic waves only occurred when fluid was forced past the bridge. In figure 3, this occurred as is evident by the slow shortening occurring in lower half. The peristaltic waves (levers are set immediately below each other) occur later in the lower half and persist longer and are not in phase with the upper. A small longitudinal slit immediately below the bridge was made in the lower half so that the pressure escaped. On raising pressure the upper half showed peristalsis, the lower half only movements due to fluid passing the bridge. In no case where the bridge was stretched sufficiently tightly to prevent the passage of fluid did the lower half respond. When, however, fluid did pass, movements occurred.

C. 2. A second method was therefore devised by W.G.M., of a similar type. A longitudinal slit surrounded with a very fine silk purse string ligature was made in the middle of a strip of guinea-pig gut some 10 to 12 cm. long. Through this slit was passed a right-angled glass cannula just sufficient to distend the gut, and the purse string ligature tied. The cannula was then fixed. The only interruption to nervous conduction was assumed to be that due to the slit and ligature. Both ends of the gut were provided with pressure bottles and attached to levers, and the pressure raised in the lower half to a just subcritical pressure. On raising the pressure in the upper half to a critical pressure a peristaltic response was obtained. The pressure was in no case kept up for long and adequate rests were given. After resting and on raising the pressure some 4 to 5 mm. higher than the critical pressure in the upper half, fluid passed round the cannula and a response was once obtained in the lower half also, figure 4. As may be seen, however, the rate in the two halves was different and in some cases as above died out in the lower half before the pressure was decreased. The third group in the lower line of the tracing was made with the original pressure.

It may be borne in mind that Henderson has shown that the rate of peristaltic movement varies with the pressure. The critical point is not precise if the increase in pressure is slow. In all these experiments the rate of rise of pressure was approximately 10 mm. in 15 seconds. If clear cut and sharp movements are given with a pressure of say 14 mm. water, a pressure some 2 or even 3 mm. lower will lead to some shortening of the longitudinal coat with irregular movements and finally peristaltic waves. However,

the frequency of these waves will be less with the lower pressure. This is well shown in figure 4, A.

These two groups of experiments (section C, 1 and 2) would go to show that the increase of pressure set up in the upper half produced peristaltic waves but that a conduction of these waves did not occur to the lower half. Trendelenburg (1911) has shown that if a central area of a strip of gut be compressed with Pean's clamp so that plexus and muscle coats are destroyed, a peristaltic wave passes the gap, thus agreeing with the results of Cannon (1912). Fleisch considered that he had shown by his experiments that an impulse passed the bridge and caused shortening of the gut beyond it even when the muscle ring held by the clamps could not shorten. This we could not confirm and the experiments with the bridge and tube methods go to show that conduction of the peristaltic impulse does not pass a mechanical obstruction which prevents the passage of fluid and a pressure change at a lower level. We do not believe that in either of these two methods the nerve connections were so damaged as to obstruct the transmission of an impulse.

To explain these facts we are inclined to assume that the impulse to peristalsis does not travel far in the plexus but only the immediately adjacent muscle fibres respond. Their contraction displaces fluid to a lower level where the internal pressure is almost sufficient to produce activity and hence the wave is reinitiated at successively lower levels in the Trendelenburg method. In the gut set up in such a method as that of Baur or Sollmann or in an essentially similar method such as we have employed, it progresses or fades out dependent on the preëxisting pressure in the lower segments.

D. The absence of inhibition. The movements of short sections of guinea pig and rabbit gut set up according to Trendelenburg were recorded with a lever which had a considerable magnification, 4 to 5 times for the longitudinal coat, and a volume recorded of small capacity so as to give relatively large movements for the circular coat. The movements were recorded on a drum whose speed could be increased. In other cases a modified method was used. The cannula in the aboral gut end was the centre limb of a T. One lateral limb went to the pressure bottle, the other to a piston recorder set above a cylindrical chamber of considerable bore. On raising the pressure fluid rose in the chamber lifting the piston recorder. At the correct pressure the pressure bottle was cut off. Fluid expelled from the gut went toward the recorder but caused little increase in pressure. It was then evident that the longitudinal coat particularly showed rhythmic movements superimposed on the peristaltic wave. The eye could readily observe that the peristaltic wave gradually spread from the upper to the lower end and relaxation only occurred after the whole strip which was only some 4 cm. long had contracted and in consequence the rhythmic waves

recorded were produced in the area immediately below the advancing peristaltic waves. That is, there was no inhibition of rhythmic movements immediately below the contracted portion of the wave, as may be seen in figure 5.

E. On the origin of the peristaltic wave. Trendelenburg method, 8.5.23. Guinea-pig gut. Jejunum in good tonus. Length 10 cm.

2:30 p.m. First tested with pressure of 15 mm. water. Rapid response in form of series of peristaltic waves running the whole length. The rise of the longitudinal lever was rapid to onset of first abrupt peristaltic contraction; 20 peristaltic waves per minute. Lowered pressure, peristaltic waves ceased. Raised pressure to 12 mm. The rise of the longitudinal lever was as rapid at first, then a slower rise of 2 mm. on tracing, then the abrupt rise of first peristaltic wave. The rises less high. This first wave did not begin at the oral end but 2 cm. from it, spread to the aboral end. As the first wave relaxed fluid surged into the gut to oral end. Looked almost as though there were a recoil wave at the oral end. Second wave began at oral end and spread more rapidly than first and was succeeded by others. Peristaltic waves 12 per minute. This corresponds closely to figure 4 (Henderson 1923) or group A, figure 4.

Guinea-pig gut. Modified Baur method, 22.7.24. Piece jejunum ileum 20 cm. long.

Inflow pressure slowly raised. Inflow pressure 10 mm. Outflow pressure 0. Cutting off flow raised pressure to 12. First peristaltic wave arose at 3 cm. from oral cannula, spread 6 cm., then died out. Relaxation slow from upper end, quiescent, marked longitudinal movements with shortening of 7 mm. flow small, 2 cc. 1 per min. roughly. Second peristaltic wave at same point spread about 10 cm. followed by third wave at about 2 cm., then one at 1 cm. and at 0.5 cm., then arrest with increasing longitudinal pendular then circular movements. The gut appeared smaller, flow slower, 1 cc. in 2 min. Then another series at 4 cm., 3 cm., 2 cm., 1 cm. roughly.

Baur method, 10.12.26. Jejunum showing good activity, 13 cm. long.

10:35 a.m. Set up.

10:47 a.m. Pressure 10 to 20. Good long peristaltic series. Gut much elongated. Long conical contraction arising at 2 cm. extending to end, then relaxation.

10:52 a.m. Lowered to 10, still good peristaltic waves.

10:55 a.m. Lowered to 8, quiescent, gut showing increasing pendular movements, then peristaltic waves.

10:58 a.m. Peristaltic waves run more slowly. A long area still progressively contracted, last 3 cm. not greatly. Upper end very complete relaxation, upper portion relaxes slightly before lower. Pressure changes at oral end of 8 to 10 mm. are caused merely by cessation of flow.

11:05 a.m. Wave running whole length but relaxes at upper before reaching lower, and a second faster moving wave seems almost to catch up to first. Relaxation then general and almost simultaneous throughout strip.
11:10 a.m. Antiperistaltic wave.
11:13 a.m. Incomplete short peristaltic waves in the oral 3 cm., lower part relaxed, quiescent, dilated.
11:18 a.m. Pendular movements more active.
11:21 a.m. Another good series of peristaltic waves.
11:23-25 a.m. Antiperistaltic wave from 6 cm. to 10 cm., then peristaltic wave arising at 1 cm.
11:57 a.m. Pressure raised to 12. Rapid flow, peristaltic waves occur.

In the Trendelenburg experiment when the pressure was low, 12 mm., the first wave did not begin at the oral end but 2 cm. from it; the subsequent waves beginning at the oral end, were transmitted to the aboral end and were of a faster rate. The increase of pressure which set up the first wave was at the rate of 10 mm. in 15 seconds. No further increase was made. This slowly increasing pressure was adequate to act as a stimulus at a point 2 cm. from the oral end. (This implies that we assume that this area was more irritable than the rest.) The peristaltic wave thus set up forced out fluid, which returned on the occurrence of relaxation, at a much more rapid rate. The rise of internal pressure was more rapid and areas of lower irritability such as in the upper 2 cm. could be stimulated. (Trendelenburg (1917) has shown that the more rapid the rise of the pressure, the less pressure required to provoke peristalsis.) Both Schneller (1925) and Trendelenburg (1917) are wrong when they assert that in the Trendelenburg method, the peristaltic wave invariably begins at the upper tied end. If the pressure movement is slow and a subcritical pressure is used, the first wave rarely arises at the upper end and the assumption made by Schneller (and Trendelenburg) that the wave arises at the upper end owing to the tonus of that area being increased due to the ligature becomes valueless. These observations, taken in conjunction with the facts reported in the subsequent experiments above (Baur method) and the facts reported by Sollmann (1928) are certainly not in harmony with Alvarez' assumption of a gradient, such that the most oral area is always the most irritable.

In the Baur gut, however, into which fluid is flowing at a low pressure, often in our experiments and those of Sollmann (1928) at a pressure inadequate to force the fluid through its whole length, the explanation must be somewhat different. The peristaltic wave beginning at a certain point cuts off any inflow past this point. Now in our experiments such cutting off of the flow caused a rise of pressure of 2 to 5 mm. of H_2O. Upper reaches of the gut would be exposed to a higher pressure than before and subsequent peristaltic waves would occur at a higher level. Further, as the gut relaxes from the oral end the fluid flows forward more rapidly than at first.

The rapid forward flow is arrested in our experiments by the slight positive pressure required to pass the lower cannula.

After a peristaltic wave or waves there is a period of quiescence succeeded by a period of increasing pendular movements. These pendular contractions of longitudinal and circular coats undoubtedly move the contained fluid to and fro. They occur constantly and are very evident in rabbit gut and usually are quite marked even in guinea-pig gut, and the impression is produced that they play some part in leading to the subsequent peristaltic activity. Possibly the pressure changes, minor though these are, may well be sufficient to set up the peristaltic response, as a change of 1 mm. from just subcritical is often sufficient.

The view we hold of the peristaltic mechanism may then be pictured as follows. Fluid reaching an area and accumulating there gradually distends the wall. If the increase is sufficiently rapid the muscle responds like smooth muscle everywhere by shortening as in figure 2, A. This is a purely muscular response. But from receptors in the muscle or elsewhere impulses pass to the ganglia cells, and if of sufficient intensity and if the ganglia are normal the cells respond by discharging impulses to longitudinal and circular coats. The longitudinal coat shows its response first. This contraction of the coats would be called by some a tonus wave but it is of a more temporary character than many tonus waves (see for example fig. 3, Henderson 1923). The initial constriction and shortening spreads to ever lower levels if the pressure changes are adequate and the ganglia cells are active. The original ring broadens to a band: the band relaxes usually from the oral end where it began. One discharge or several discharges rapidly following each other exhaust the nerve cells, and they must be allowed to recover. Or to use v. Uexküll's analogy, the sensory impulses charge the tonus reservoir (in the cells). This in turn discharges tonus in a certain quantity under a certain tonus pressure (Tonusmenge and Tonusdruck). The reservoir becomes emptier, the discharge less effective (note the irregular weak waves at the end of a series). The gut becomes quiescent suggesting that the discharge of the reservoir (nerve cells) also affects the rhythmic movements. It seems to be the case that even the rhythmic movements are less marked when gut tonus is low. Gradually the myogenic rhythmic movements resume, the local reservoir is refilled, the tonus becomes somewhat greater and the movements more ample, till finally conditions are ripe for a new peristaltic discharge. The more rapid the rise of pressure the more rapid the response and this may lead to the response arising from an area which was not sufficiently irritable to respond to a slower pressure increment.

F. Conditions in the mechanism necessary for peristalsis. In many experiments with guinea-pig gut I have noticed that when the gut appeared platulous and relaxed, peristalsis was only produced with high pressures

and the waves were few in number before ceasing, and spread only over short lengths and possibly did not completely occlude the lumen. In the rabbit the same was observed, peristalsis was with difficulty produced in the patulous gut even when the rhythmic movements of the longitudinal coat were ample, a protocol of such a piece of gut is given above, 5.5.28 (section B 2). The effect of adrenalin, of pilocarpine and the ready distensibility of the gut indicate low tonus on both the longitudinal and circular coats. Increase of internal pressure in this experiment caused a considerable shortening of the longitudinal coat which was not mechanical (dead gut never gives such a shortening). There was a definite increase of longitudinal tonus but this did not spread apparently to the circular coat and no peristaltic waves occurred. Such patulous strips do not show peristalsis. On adding nicotine in this case to the gut without internal pressure there was a great longitudinal shortening of very brief duration and with the longitudinal relaxation the circular coat contracted. (The tonus appeared to have passed from the circular to the longitudinal coat.) The circular coat showed rhythmic activity which it had not previously done; under pressure it became more active but the longitudinal coat was less active under both conditions.

A somewhat similar state of affairs appears in the following experiment.

Trendelenburg method, 22. 3. 28. Rabbit gut, methane and intraspinal anesthesia. Jejunum, 10 cm. long.

2:10 p.m. Pressure 0. Active pendular movements in both coats. Pressure raised to 10 mm., shortening of longitudinal coat, increase in diameter, peristalsis. Lowered pressure.

2:18 p.m. Pilocarpine 1:120,000, shortening of longitudinal coat relatively slight, great decrease in diameter.

2:20 p.m. Raised pressure to 10 mm. no inflow, 20 mm. a little shortening of longitudinal coat. No inflow into gut. Pressure 30 mm., still no inflow. Gut small cord like, had shortened a little, more length now 8.2 cm.

2:30 p.m. Pressure raised to 40 mm. Small inflow, gut a little dilated, slightly shorter. Apparently two peristaltic waves progressing only 2 cm. to reach cannula, and then gradual dilatation.

The above experiments which are typical of many others we explain as follows. Before the pilocarpine the tonus of both coats was good. The gut before removal had showed active rhythmic movements. After pilocarpine there was a relatively great increase in circular tonus. As the pressure bottle was raised the pressure exerted at the aboral (cannula) end on the gut caused a local increase in tonus, largely in the circular coat in this case, while usually it is most evident in the longitudinal coat. The increased circular tonus prevented further inflow and only slowly with high pressure was relaxation enough secured to enable a peristaltic movement to be evident and this very slow distention seems to lower gut tonus. Just as

v. Uexküll (1904) and others have found that slow stretching of smooth muscle causes a loss of tonus.

The impression gained from many experiments of these types has led to the conclusion that there must be a certain amount of tonus present in the gut strip and that such tonus must be present in both coats, and further, that there must be a certain balance in the tonus of the two muscular coats.

G. On the nervous mechanism. We have long been impressed by the fact that the response of the isolated but depended upon the state of the gut before removal. The mere opening of the abdominal cavity, when the sensory supply to the cord and the efferent sympathetic supply is intact, leads to a reflex inhibition. This is evident from the work of Bayliss and Starling, and especially from the papers of Wagner (1923), and Markowitz and Campbell (1927). We therefore agree with Thomas and Kuntz that there is a definite reflexly produced inhibition of rhythmic movements and tonus. We further believe that the low tonus thus produced remains after the gut is removed from the body.

It has further long been known that atropine in relatively small doses decreases gut tonus but that in even large doses does not interfere with the increase in size of rhythmic movements produced by vagal stimulation (Bayliss and Starling, 1899; Cushny, 1910; Henderson, 1923b). Normally, i.e., without atropine, vagus stimulation increases both tonus and movements. Evidently the vagus endings for tonus are readily depressed by atropine, those for contractility are not.

According to Thomas and Kuntz (1926a) small doses of nicotine decrease the effect of vagus stimulation on both tonus and rhythmic movements. If I understand them correctly they assume that this is due to a stimulation of sympathetic cells by the nicotine setting up a maintained inhibition. This seems to me entirely plausible. Larger doses of nicotine lead to an even greater or more readily produced vagus effect on tonus and movements. Presumably the sympathetic cells are now depressed and the vagus cells have not been depressed as yet since still larger doses do depress the vagus.

If we accept the usual pharmacological views as to the sites of action of these drugs and try to form some picture of the sites of their action in the histological structure of the gut, it seems that a diagrammatic scheme such as figure 6 will serve.

The vagus is shown with two cells A and B. The post ganglionic fibres from A pass directly to the muscle cells. These endings are resistant to atropine. The endings from B pass to the intramuscular nerve net of Tiegs lying in the muscle coats. Their function is to distribute tonus to the new work. These endings are readily depressed by atropine. The intracellular nerve net is introduced to form a connection between the two coats so that their rhythmic contractions are synchronous. The sympathetic post

ganglionic fibres pass to the muscle cells and depress their tonus, abolish it, and movements cease. I have inserted cells C which like the vagus cells are contained in the plexus of Auerbach. They receive sensory impulses from muscle or from mucosa and the second cell distributes its impulses to short sections of the nerve net. Small doses of nicotine stimulate the sympathetic cells and decrease the muscle tonus of both coats. Rhythmic movements are small or absent, peristalsis also. Larger doses depress the sympathetic cells but not vagus cells. Peristalsis should be present but I have failed to find it. Possibly because I have not been fortunate or possibly because vagus cell B, being depressed by this dose though A is not, has upset the distribution of tonus to the nerve nets. Tonus waves occur, but orderly peristalsis does not. It may be that cell C in the reflex arc is also depressed. Larger doses of nicotine depress vagus cell A and also reflex arc cell C.

The main conclusions are drawn from work on the rabbit and guinea-pig gut when isolated. Experiments with the isolated gut of other animals have not as yet yielded results which we consider binding as gut from animals with thicker muscular coats is more difficult to maintain in a satisfactory condition.

The mechanism and character of the antiperistaltic movement which has been seen in both Trendelenburg and Baur preparations has not been discussed as insufficient facts are at hand to enable this to be done with profit, but we believe that an explanation not incompatible with the above may be given. Further studies are in progress to extend these conclusions to that of other animals both intact and isolated.

SUMMARY

1. Evidence has been presented to show that nerve cells are concerned in the peristaltic mechanism.

2. That the peristaltic mechanism consists of reflex arcs which are short.

3. That slight increments in pressure may be sufficient to elicit the peristaltic wave.

4. That the peristaltic wave normally consists of a short lived increase of tonus in both coats.

5. That the peristaltic wave is not preceded by an area of inhibition.

6. That the peristaltic wave arises at a point where the pressure changes are most effective and that this point may change its position dependent on the changes in pressure produced by previous waves or possibly by rhythmic movements.

7. That an equitable distribution of tonus in both coats and to both coats is necessary for the production of peristalsis.

8. The nervous innervation is discussed.

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THE USE OF NITROGEN FOR DETERMINING THE CIRCULATORY MINUTE VOLUME

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Bornstein in 1910 attempted to determine the blood flow through the lungs of man by estimating the amount of nitrogen given off when pure oxygen is breathed. The principle of his method depends upon the determination of the rate of elimination or absorption of an indifferent gas (e.g., nitrogen) across the pulmonary epithelium. Assuming the blood leaving the lungs to be in equilibrium with the pulmonary gases, and knowing the solubility of the gas in blood, one can calculate the amount of blood passing through the lungs in unit time. This principle is the basis of subsequent methods for determining the cardiac output by the use of foreign gases. The use of nitrogen was found unsatisfactory by Krogh and Lindhard (1912), who then developed their nitrous oxide method on the same general principle.

Bornstein (1920) has recently modified his method in certain details. In its most recent form it is as follows: Two rubber bags are filled with oxygen. The larger (bag 1) holds 8 to 10 liters and is used in the preliminary period; the smaller (bag 2) holds about 3 liters and is used in the main experiment. The bags are connected through a U-tube containing potassium hydroxide with a two-way tap and mouthpiece. The subject expires to the residual air, breathes at first deeply then normally from the large bag for 70 seconds. After another expiration to the residual air the subject is transferred to the second bag for three minutes. At the end of this period a final expiration to the residual air concludes the experiment. The volumes of gas in the bags at the beginning and end of the experiment are carefully measured as well as the volume of the U-tube and tap. The nitrogen percentages of the gas in the bags at the beginning and end of the experiment are also determined. The volume of residual air in the lungs (determined from data on the large bag) and the above data allow a calculation of the amount of nitrogen eliminated from the body during the three minutes' rebreathing into the smaller bag. The mean tension of nitrogen in the alveolar air (average of that of bag 1 and bag 2) and the assumption of a tension of 80 per cent of an atmosphere in the blood coming

to the lungs enables one to calculate the elimination of nitrogen per 100 mm. tension difference per minute. This value is increased by 5 per cent to correct for the drop in nitrogen tension in the venous blood from the time of the first circulation. Finally, using Buckmaster and Gardner's (1911–12) value for the solubility of nitrogen in arterial blood, he calculates that for 100 mm. tension difference each cubic centimeter of nitrogen eliminated corresponds to a blood flow of 671 cc.

The method of Bornstein is rather laborious and does not inspire confidence in its accuracy. The main criticisms of the method which may be made are 1, the analytical methods available for estimating nitrogen are not sufficiently accurate; 2, the solubility of nitrogen in blood is not known accurately and there is a question of its being entirely in physical solution; 3, there is no direct proof that equilibrium is attained for nitrogen between blood and alveolar air; 4, an error may be introduced by the elimination of nitrogen from the tissues of the lungs, bronchi, trachea and mouth; 5, there is no proof that the correction of 5 per cent gives the nitrogen elimination during the first circulation of blood, and 6, there is no proof that the blood flow is unchanged during the experimental procedure of rebreathing.

In a study of the use of the rate of elimination of nitrogen for determining the circulation rate, we have investigated these possibilities of error and are able to give experimental data on these points. Although an analytical method for nitrogen which is about ten times as accurate as those in general use has been devised, apparently unavoidable difficulties have prevented the development of a satisfactory method for determining blood flow by the use of nitrogen. Nevertheless, the information which we have accumulated in relation to the whole question of the elimination of nitrogen from the blood would seem to be important enough to warrant a brief account of our investigation.

Method of analysis. The Van Slyke-Neill (1924) manometric gas analysis apparatus can be used very successfully in determining small quantities of nitrogen in a mixture containing oxygen and carbon dioxide. After evacuating the pipette and setting the mercury level at the 50 cc. mark, the manometer is read (p_0). A sample of 30 to 50 cc. of the gas is now introduced, the mercury level set at the 50 cc. mark and the manometer again read (p_1). Five cubic centimeters of an air-free mixture of potassium hydroxide, sodium hydrosulphite and sodium β-anthraquinone sulphonate (Fieser, 1924)[1] are now introduced and the apparatus shaken for 2 or 3 minutes. A reading is now taken with the liquid meniscus at the 2

[1] This mixture is prepared as follows: To a cold solution of 14 grams of sodium hydroxide in 100 cc. of water are added 16 grams of sodium hydrosulphite (Dupont) and 2 grams of ordinary commercial "silver salt" (sodium anthraquinine β-sulphonate). The final solution is filtered through cotton, evacuated to free it of the dissolved gases and kept over mercury out of contact with air.

cc. mark on the pipette (p_2). The apparatus is again partially evacuated and the reading repeated after 1 minute shaking. When a constant reading has been obtained the nitrogen gas is expelled and another reading taken with the liquid at the 2 cc. mark (p_3). The percentage of nitrogen is then calculated from the formula: $\frac{p_2 - p_3}{(p_1 - p_0) \times 25}$. If the first reading ($p_0$) is made with a trace of lactic acid solution present in the pipette, the vapor tensions need not be considered in any of the readings unless marked changes in temperature have occurred during the analysis. The apparatus is washed as usual with water and then with dilute lactic acid solution and is ready for another determination. The accuracy of the analysis of nitrogen by this method is about $\pm$ 0.003 per cent of nitrogen. Three duplicate analyses of an oxygen cylinder gave 0.867, 0.867, 0.864 per cent nitrogen and on another tank the following values were obtained; 0.483, 0.483, 0.482, 0.476 per cent nitrogen.

Solubility of nitrogen in human blood. Previous determinations of the solubility of nitrogen in blood have given values distinctly higher than those expected from the solubility in water (Bohr and Henriques, 1897; Van Slyke and Neill, 1924). The amount of nitrogen present in blood was determined on a number of normal individuals. The blood was drawn from an arm vein and analyzed in the Van Slyke-Harington (1924) blood gas apparatus. The determinations were made with 5 cc. samples, the oxygen and carbon dioxide being removed by absorption with the anthraquinone-hydrosulphite mixture. In order to determine whether equilibrium existed between the nitrogen tension of the blood and that of alveolar air some of the samples were equilibrated in tonometers at 37.5° and the nitrogen content subsequently redetermined.

The first series of determinations were made on oxalated blood, the material being collected directly in calibrated pipettes provided with a ground glass tip to receive the needle. The oxalate was dried in a thin film on the walls. The average value obtained in this series of determinations was about 1.0 vol. per cent. It appeared possible that these results might be too high on account of air being included by the blood in its passage over the irregular surfaces presented by the oxalate crystals in the pipette. Another series of estimations were now made in which the blood was transferred (after removal of the needle) directly to the blood gas apparatus. In this series of determinations no anti-coagulant was introduced into the pipette, the sample being immediately transferred to the blood gas apparatus which contained the oxalate. It will be seen that the values average 0.9 vol. per cent, individual determinations ranging between 0.85 vol. per cent and 0.95 vol. per cent (table 1). Equilibration of the samples with air at 37.5 per cent gave nitrogen values of the same magnitude. A few determinations showed no changes in the nitrogen content

of the blood (within the errors of the analytical procedures) as a result of smoking or of fat ingestion. The average result obtained (0.90 vol. per cent) is still slightly higher than that which one would expect (0.83 vol. per cent) from the solubility in water and Bohr's (1905) finding that the solubility of a chemically inert gas is 92 per cent of its solubility in water.

TABLE 1

Solubility of nitrogen in human blood

SUBJECT	CONDITION OF EXPERIMENT	BLOOD NITROGEN
		vol. per cent
C.	Venous blood	0.89
	Equilibrated with air	0.85
H.	Equilibrated with air	0.89
	Venous blood	0.92
	Venous blood 2 hours after ingestion of 250 cc. of 40 per cent cream	0.96
	Venous blood	0.93
	Equilibrated with alveolar air	0.90
G.	Venous blood	0.83
Ha.	Venous blood	0.90
	After smoking	0.95
	Equilibrated with air	0.89
	Venous blood	0.88
W.	Venous blood	0.91
S.	Venous blood	0.89
M.	Venous blood	0.95
	Equilibrated with air	0.78
P.	Venous blood	0.86
	After smoking	0.89
R.	Venous blood	0.93
	Arterial blood	0.90

Equilibrium between alveolar air and arterial blood. In order to determine if equilibrium is attained between the alveolar air and arterial blood when pure oxygen is breathed, the content of nitrogen in arterial blood under these conditions was determined. Blood was drawn from the radial or brachial artery at various times after beginning to breathe oxygen. Results are given in table 2. The last four determinations were done using oxalate

in the pipettes and are probably 0.1 vol. per cent too high, due to the inclusion of air as discussed above. In general, it may be concluded that equilibrium is attained.

Method for determining rate of nitrogen elimination from the body when pure oxygen is inspired. Several methods of determining the rate of elimination of nitrogen from the body when oxygen is breathed were tried. Some were quite successful when the nitrogen elimination was determined over several minutes, but the determination of the rate over fractions of a minute presented difficulties. The following method was found suitable. The apparatus used is pictured in figure 1. A is a tank of compressed oxygen connected through a reducing valve to a rubber bag *B* of about 20 liters capacity. The bag *B* is connected by means of a three-way tap *C* with a respiratory valve (Lovén type) *D*, the expiratory side of which is connected to another three-way tap *E*, so that the expired air can pass either through the rubber tubes *F* or *G*. Both of these tubes

TABLE 2

Nitrogen content of arterial blood while breathing oxygen

SUBJECT	TIME SAMPLES—MID-POINT	BLOOD NITROGEN
		vol. per cent
S	2 minutes	0 04
W	5 minutes	0 08
P (diabetes)	19 seconds	0 162
H (psychoneurosis)	40 seconds	0 11
B (rheumatism)	4 minutes 5 seconds	0 12
Ca (scleroderma)	2 minutes 30 seconds	0 15

have Sadd flutter valves on the end, and tube *G* passes into a rubber bag *H* of about 8 to 10 liters capacity. At *I*, *J* and *K* mercury sampling tubes are attached and the connections filled with mercury.

To carry out an experiment the whole system is first washed free of air with oxygen from the tank and bag *B*. This is best accomplished, after washing bag *B* several times through tube *F*, by filling and emptying bag *H* about five times. A suction pump attached to the three-way tap on the sampling tube at *J* empties the bag *H* very rapidly. The tap *C* is turned to communicate with the outside air; tap *E* connects with tube *F*; and bag *H* is empty. The subject now clips his nose, places his mouth on the mouth piece attached to valve *D* and breathes normally outside air, inspiring through *C* and expiring through tube *F*. The bag *B* being filled with oxygen the subject expires to his residual air through tube *F*, tap *C* is turned and several rather deep respirations are taken and then normal breathing is resumed. At an appropriate time an expiration to the residual air is made through tube *F*, the tap *E* turned and after fifteen

seconds breathing into bag *H* a final expiration to the residual air is made into bag *H*. After each forced expiration samples of alveolar air are taken at *I* and *K* respectively, and after the final forced expiration a sample of gas from bag *H* is taken at *J*. The volume of gas in *H* is then determined with a wet meter. The time of the start of the experiment (turning tap *C*) as well as those of the ends of the two forced expirations are accurately noted with a stop-watch with split second hand.

The three samples (alveolar I taken at *I*, alveolar II taken at *K* and bag sample taken at *J*) are analyzed for their nitrogen content. The nitrogen content of the tank *A* is accurately determined for each new cylinder. The calculation can best be illustrated by describing it for a typical experiment.

Subject A. G. Increased breathing from 0–8 seconds, alveolar I taken at 21.5 seconds, alveolar II at 42.5 seconds. Duration of collection of expired air 21 seconds, mid-point 36.8 seconds. Contents of bag *H* at end of experiment was 5.60 liters at 23° and 756.5 mm. (at 23°). Volume at S. T. P. = 4.97 liters. The difference in volumes of inspired and expired air, due to the R.Q. not being 1.0, has been neglected,

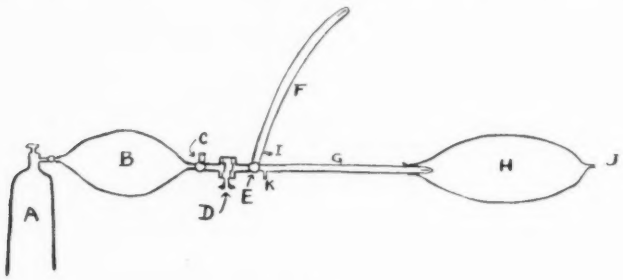

Fig. 1

but involves a negligible error. Bag sample contained 1.330 per cent N_2, oxygen tank, 0.770 per cent, difference 0.560 per cent. $4.97 \times 5.60 = 27.83$ cc N_2. This value (27.83 cc. N_2) must be corrected for the difference in the amount of nitrogen in the lungs at beginning and end of the collection of expired air, and also for the difference in nitrogen content of tube *G*. Residual air of subject was 1.07 liters (at S. T. P.) including dead space of mouth piece, valve D and tube to tap *E*. Volume of tube *G* was 0.25 liter. Alveolar I contained 2.220 per cent and alveolar II, 1.640 per cent N_2; difference = 0.580 per cent. $1.07 \times 5.80 = 6.2$ cc. Difference between second alveolar sample and tank = 0.870 per cent N_2. $0.25 \times 8.70 = 2.18$ cc. The first of these corrections (6.2 cc.) must be subtracted and the second (2.18 cc.) added to 27.83 cc. to get the amount of nitrogen eliminated from the blood. Total amount of nitrogen eliminated from the blood from 21.5 to 42.5 seconds after commencing to breathe oxygen, therefore, equals 23.81 cc. Rate of elimination per minute $= 23.81 \times \frac{60}{21} = 68.0$ cc.

Error introduced by nitrogen elimination from tissues of the respiratory tract. That the nasal cavity can act as a more or less closed cavity to and

from which diffusion of gases in the mouth is very slow can be demonstrated by a very simple experiment. If one exhales smoke through the nose, holds the nose while smoke is still issuing, then forcibly ventilates the lungs for several seconds, smoke can still be blown from the nostrils when the fingers are removed. The magnitude of this error can be determined by comparative experiments, in one of which the nose is clipped as usual, while in the other, the nose is held with the fingers and inspiration taken by mouth and expiration made through the nose several times. The error in determining the rate of nitrogen elimination during the first minute may be considerable. The procedure of washing the nasal cavity with the first few breaths of oxygen was, therefore, adopted in all the data reported here.

The elimination of nitrogen from the tissues of the mouth was studied as follows. Breathing was carried out as usual with nasal wash from the bag and tank of oxygen for 18 seconds. The mouth was then transferred to a bag containing about 3 liters of oxygen and rebreathing carried out for about 10 seconds, when a forced expiration was made into the bag with the cheeks expanded. The bag was then shut off from the mouth. After holding this position for 20 seconds samples of gas were drawn from the mouth and the bag. The mouth sample contained 0.11 per cent N_2 more than the bag sample and if one assumes the capacity of the mouth to be 200 cc. the maximum elimination of nitrogen would be 0.22 cc. in 20 seconds or at the rate of 0.66 cc. per minute. This last figure shows that a negligible error is introduced into our experiments from this source.

Rate of nitrogen elimination from the blood at different times. The rate of elimination of nitrogen has been carefully studied at different time intervals after the start of oxygen breathing in one individual. In figure 2, the results for this individual (A. G.) are plotted as cubic centimeters of nitrogen eliminated per minute from the blood against time in seconds after commencing to breathe oxygen. Determinations were made on different days several hours after breakfast. A smooth curve drawn through the points may be considered in three parts: a period of constant nitrogen elimination which lasts for about 40 seconds, a period of rapidly decreasing rate of elimination which persists for about the next 80 seconds and a final period in which the rate of elimination is decreasing very slowly. Our interpretation of the curve is that the first portion showing a constant rate means that the rate of elimination of nitrogen is constant until the blood has made one complete circulation. To interpret the second phase of the curve, the period of rapidly decreasing rate, one must consider the fact that at rest a large proportion of the total output of the left ventricle is going through the viscera and brain which comprise only about 10 to 15 per cent of the body weight, while a relatively small amount of blood is flowing to the rest of the body—skin, skeletal muscle, fat and

bone (which comprise 85 to 90 per cent of the body weight). The reservoir of nitrogen in the tissues of the viscera and brain will, therefore, be very quickly depleted and the large amount of venous blood returning from these regions to the right heart will soon be almost depleted of nitrogen. This returning blood from the viscera will mix with a smaller quantity of blood from the periphery of the body which has still nearly its full quota of nitrogen. This is shown in the results obtained on venous blood taken from the arm about 1 minute after breathing oxygen (0.78 vol. per cent instead of 0.90 vol. per cent). The phase of rapidly decreasing rate of elimination consequently represents the time during which the visceral organs and brain are being rapidly freed of their nitrogen. The third phase of very slowly decreasing rate of elimination represents the time when the viscera are practically free of nitrogen and the returning venous

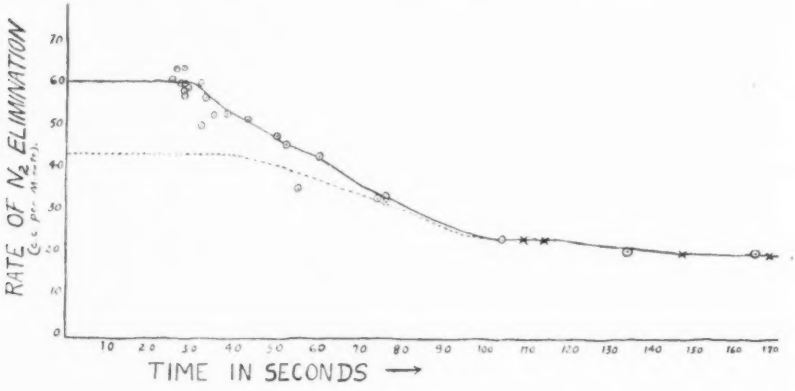

Fig. 2

blood from the large reservoir of the muscles, skin, fat and bones is losing its nitrogen very slowly.

A little consideration indicates that such a curve as we have drawn in figure 2 is a composite or average of a large number of somewhat similar curves which represent the rate of elimination of nitrogen from the various organs and tissues of the body. Since the blood-flow through different organs varies widely, the shape of their curves will differ considerably. A change in the distribution of blood in the body will consequently affect the general shape of the average curve which alone we can at present determine. Hence any attempt to extrapolate backward to get the height of the curve during the first circulation will be impossible for different conditions of blood distribution. The only feasible plan is to attempt to measure the rate of nitrogen elimination during the time when

the curve is a plateau, which means that the determination must be completed before any appreciable amount of blood has recirculated.

Effect of experimental procedure on blood flow through the lungs. Using the apparatus and method already described for determining the rate of nitrogen elimination, we have made determinations of the rate of nitrogen elimination during the first period of the curve when the rate is constant. With the precaution of being careful to wash the nasal cavity, the nitrogen elimination from the blood has been determined during the period of 20 to 35 or 40 seconds after commencing to breathe oxygen. The figure expressing the number of cubic centimeters of nitrogen eliminated per minute during this time divided by 9 gives the blood flow (in liters per minute) through the lungs during the experiment.

TABLE 3

Apparent circulatory minute volume of subject: A. G.

DATE	N_2	C.M.V.	DATE	N_2	C.M.V.
1926	*cc. per minute*	*liters*	*1926*	*cc. per minute*	*liters*
June 5	52	5.8	November 4	62	6.9
June 5	55	6.1	November 4	57	6.3
June 8	69	7.7	November 4	61	6.8
June 8	70	7.8	November 6	61	6.8
June 8	70	7.7	November 9	59	6.6
June 9	54	6.0	November 13	66	7.3
June 9	56	6.2	November 16	60	6.7
November 2	59	6.6	November 18	66	7.3
November 2	61	6.8	November 19	64	7.1

The above results have been obtained on one subject (A. G.) after sitting for about 15 minutes in a chair before the experiments. All determinations were made in the forenoon about 3 to 4 hours after breakfast.

It is evident from these results that very satisfactory duplicate determinations of the circulatory minute volume can be made at the same sitting, but that some variation occurs on different days. Considering the fact that the individual was not in the basal condition, the general constancy of the values is quite remarkable. However, subsequent experiments and determinations on other individuals have led us to doubt the accuracy of the whole method. The above table is given to show that consistency of results is no valid criterion of the accuracy of any blood flow method. On other individuals, not as well trained in carrying out the experimental procedure, the results obtained on the same day were much more variable.

Subsequent work has indicated definitely that the blood flow is increased during the experimental procedure, by the increased breathing in the

preliminary period of 20 seconds. Unfortunately, it is extremely difficult to make a correction for this increase.

If air is breathed instead of oxygen and the experiment performed just as in the blood flow experiment, it is found that the oxygen absorption, during the experiment, is increased over the value determined in the usual manner just before the experiment. Moreover, a determination of the oxygen and carbon dioxide as well as the nitrogen percentages of the samples obtained in a blood flow experiment shows that the oxygen absorption during the experiment is higher than the values obtained by ordinary methods just before the experiment. Although there is no doubt that the oxygen absorption is increased during the experiment, it is impossible to determine accurately the magnitude of this increase for the following reason. All three gases, oxygen, carbon dioxide and nitrogen, are being exchanged across the pulmonary epithelium during the experiment and unless the respiratory quotient is unity, a volume change between the inspired and expired air occurs which cannot be corrected for. This volume change produces about five times as great an error in the calculation of the oxygen absorption when pure oxygen is being breathed as under ordinary conditions when atmospheric air is inspired. Since during the 35 or 40 seconds of the experiment the oxygen content of the mixed venous blood can be considered as constant, an increased oxygen absorption can only mean an increased blood flow through the lungs due to the experimental procedure. An accurate determination of the volumes of the inspired as well as of the expired air would allow the calculation of this correction.

Numerous determinations of the blood flow on the subject, A. G., by another method (Marshall and Grollman, 1928) have shown that this subject increases his blood flow about 40 per cent by forcibly rebreathing from a bag. Assuming an increase of the same order to have occurred in the nitrogen experiments cited above, his corrected blood flow would be about 4.9 liters as an average value, with a variation from 4.1 to 5.5 liters. This is of the same order of magnitude as we have obtained for this individual under non-basal conditions by other methods.

Taking 4.9 liters as the blood flow under normal conditions we can construct the hypothetical curve (drawn dotted in fig. 2) which represents the rate of nitrogen elimination from the body on breathing pure oxygen when conditions are normal. That the third phase of the curve would be the same in the hypothetical curve as in the constructed one is shown by the fact that the four points plotted as x were determined by another method which involved no forced breathing.

SUMMARY

A study has been made of the use of the rate of nitrogen elimination for determining the circulatory minute volume in man. The various assumptions underlying the method have been critically examined. The method of Bornstein even in its latest form has been found to be based on incorrect assumptions. However, even when these objections have been overcome the method remains impracticable for determining the circulatory minute volume.

The rate of elimination of nitrogen from the body has been determined and the significance of the curve of elimination discussed. The solubility of nitrogen in human blood was found to be only slightly greater than would be expected from its solubility in water.

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THE TIME NECESSARY FOR REBREATHING IN A LUNG-BAG SYSTEM TO ATTAIN HOMOGENEOUS MIXTURE

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The problem of the mixing of gases in the lungs and the factors determining this mixing have been investigated by many workers. A systematic study has been recently reported by Lundsgaard and Schierbeck (1923), where references to the earlier work may be found. They concluded that results obtained with hydrogen could be extended to other gases (oxygen, nitrous oxide, and carbon monoxide). Three main factors presented themselves to these investigators for study; viz., the amount of air in the lungs after expiration, the depth of rebreathing, and the number of rebreathings. By rebreathing from a rubber bag, containing 2 to 2.5 liters of a hydrogen-air mixture, and starting from the residual air of the subject, they found that three respirations were sufficient for attaining mixture in the two subjects studied. Regarding the rate of respiration, which in most of these experiments was between 10 and 15 per minute, they state, "a few experiments were performed in order to determine quantitatively the influence of the rate. We did not arrive at definite results. Our impression was that rate may be varied considerably without any appreciable influence on the result."

In connection with the problem of the determination of cardiac output in man, as reported elsewhere (Marshall and Grollman, 1928), it was found that under the conditions where Lundsgaard and Schierbeck's figures would indicate the attainment of mixture in 3 respirations, such mixture did not result with even 8 or 10 respirations. Therefore, we decided to reinvestigate the factors concerned in mixing under the conditions of our experiments. Our results indicate clearly that the rate of respiration has a pronounced influence on the number of rebreathings necessary for mixture; or, as we prefer to express it, *there is a minimum time of rebreathing necessary to obtain homogeneous mixture regardless of the number of rebreathings.* Since mixture in a lung-bag system is an important desideratum for many types of physiological experimentation, we have briefly reported our results in this communication.

METHOD. The method used in this study was similar to that employed by Lundsgaard and Schierbeck. The arrangement of the apparatus is

shown in the accompanying sketch (fig. 1). A rubber bag,[1] *C*, was attached to one end of an aluminium valve, *B*, the bore of which was 1 inch in diameter. To the other end was attached a rubber mouthpiece, *A*. The subject, having been seated for about five minutes, after applying a nose-clip, was instructed to expire to residual air and then to apply his mouth to the mouthpiece. The tap, *B*, was then turned to connect the subject with an approximately 5 per cent mixture of hydrogen in air, previously introduced into the rubber bag, *C*. The subject then rebreathed from the rubber bag, emptying the bag with each inspiration and expiring

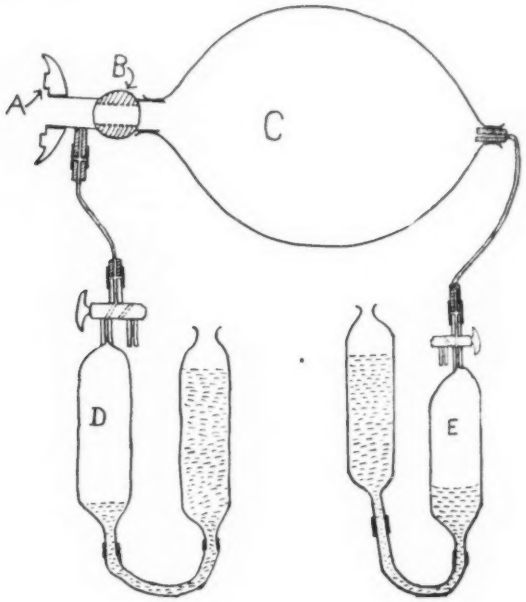

Fig. 1

deeply into the bag with each expiration. After a certain time, noted by a stop watch, and just before the end of an expiration (but while the subject was still expiring), the tap, *B*, was turned so as to disconnect the subject from the bag. A sample of the alveolar air was then instantaneously taken by opening the stopcock on the previously evacuated sampling tube, *D*, the subject retaining his mouth on the mouthpiece *A*, during this procedure. Another sample, designated "bag" sample was then drawn

[1] The size of the rubber bag should be such that the capacity is only slightly greater than the volume of gas used

into the sampling tube E by lowering the reservoir connected with it. The sampling tubes, D and E, were connected to the aluminium valve B and to the bag C, respectively, by means of lead tubing, 15 cms. long and 0.5 mm. in internal diameter. These were soldered into brass plugs as indicated in the figure. The volume of the dead-space of the system, which consisted of the mouthpiece plus the projection from the valve B upon which the mouthpiece was placed, was 40 cc.

The samples of gas were analyzed for hydrogen by burning over a heated platinum spiral. Twenty-five cubic centimeter samples were used for the analyses and were carried out in an apparatus similar to that described

TABLE 1

Subject, A. G. Vital capacity 3.7 liters; residual air, 1.0 liter

VOLUME IN BAG	NUMBER OF RESPIRATIONS	TIME OF REBREATHING	DIFFERENCE BETWEEN HYDROGEN CONCENTRATION IN ALVEOLAR SAMPLE AND IN BAG SAMPLE
liters		*seconds*	*per cent of hydrogen*
1.8	3	9	−0.03
1.8	4	12	0.00
1.8	9	9	−0.01
2.4	2	10	−0.06
2.4	3	9	−0.06
2.4	3	12	+0.01
2.4	3	12	+0.01
2.4	3	15	+0.01
2.4	4	12	+0.01
2.4	5	10	−0.01
2.4	5	15	+0.01
2.4	6	10	−0.05
2.4	6	12	+0.01
2.4	7	7	−0.03
2.4	9	9	−0.02
6.0	9	22	+0.01

by M. Krogh (1914–15) and used in the ethylene analyses described elsewhere (Marshall and Grollman, 1928). The analyses were accurate to ± 0.004 per cent of hydrogen.

RESULTS. The results of the experiments are tabulated in tables 1 to 4. The first three tables give the results obtained on three trained subjects. In the first column of these tables are given the volumes of gas which were introduced into the bag. The second column gives the number of complete respirations (inspiration plus expiration) which were taken before the valve was closed and the samples taken for analysis, as described above. In column III are given the times which elapsed from the opening

TABLE 2

Subject, E. M. Vital capacity 4.6 liters; residual air, 2.0 liters

VOLUME IN BAG	NUMBER OF RESPIRATIONS	TIME OF REBREATHING	DIFFERENCE BETWEEN HYDROGEN CONCENTRATION IN ALVEOLAR SAMPLE AND IN BAG SAMPLE
liters		*seconds*	*per cent of hydrogen*
1.8	4	12	−0.11
1.8	5	15	−0.04
2.4	3	15	−0.07
2.4	4	12	−0.07
2.4	4	15	−0.04
2.4	4	15	−0.03
2.4	4	20	+0.04
2.4	5	15	+0.01
2.4	5	15	+0.03
2.4	6	12	−0.03
2.4	7	12	−0.05
2.4	7	16	+0.01
3.0	4	12	−0.05
3.0	5	15	+0.03
4.0	4	12	−0.08
4.0	5	12	−0.03
4.0	5	12	−0.03
4.0	5	12	−0.04
4.0	5	15	+0.01
6.0	10	26	−0.02

TABLE 3

Subject, M. D. Vital capacity 4.8 liters; residual air, 1.5 liters

VOLUME IN BAG	NUMBER OF RESPIRATIONS	TIME OF REBREATHING	DIFFERENCE BETWEEN HYDROGEN CONCENTRATION IN ALVEOLAR SAMPLE AND IN BAG SAMPLE
liters		*seconds*	*per cent of hydrogen*
1.8	5	15	−0.06
2.4	4	12	−0.01
2.4	4	15	−0.01
2.4	5	12	−0.02
2.4	5	15	+0.01
2.4	6	15	+0.05
2.4	7	14	+0.05
3.0	5	12	−0.01
3.0	5	15	+0.07
3.0	7	15	+0.04
3.0	9	23	−0.10
6.0	11	28	+0.01

of the valve *B* to its closure; *i.e.*, the time during which the rebreathing was continued.

In the last column are given the differences obtained by subtracting the concentration of hydrogen found in the bag sample from that in the alveolar sample. As long as mixture between the lungs and the bag system is incomplete, the concentration of hydrogen in the bag will exceed that in the alveoli and the difference, as given in the last column of the tables, will be negative. When mixture in the lung-bag system is complete, this

TABLE 4

Mixing experiments on untrained subjects; volume in bag 2.4 liters

SUBJECT	VITAL CAPACITY	NUMBER OF RESPIRATIONS	TIME OF REBREATHING	DIFFERENCE BETWEEN HYDROGEN CONCENTRATION IN ALVEOLAR SAMPLE AND IN BAG SAMPLE
	liters		*seconds*	*per cent of hydrogen*
H. J. ♂	5.2	6	12	−0.03
		7	15	+0.09
P. A. ♂	5.7	6	12	−0.03
		8	15	+0.07
F. C. ♀	4.0	5	12	−0.06
		6	15	+0.05
P. K. ♂	6.4	5	12	−0.02
		5	15	+0.02
T. R. ♂	5.2	5	12	−0.01
		5	12	−0.01
		7	16	+0.04
E. H. ♂	5.4	7	15	+0.01
J. D. ♀	3.7	7	12	+0.01
		7	15	+0.05
R. F. ♂	4.3	5	12	−0.04
		10	15	+0.01
E. B. ♀		6	12	−0.07
		10	16	+0.05
W. D. ♂	4.2	8	12	+0.05
		6	15	+0.01

difference will be positive due to the changes in volume accompanying a respiratory quotient that is less than unity and because of the slight amount of hydrogen that is absorbed through the lungs during the experiment (Krogh and Lindhard, 1917). The attainment of a positive result, as indicated in the last columns of the tables shows, therefore, the attainment of mixture. That this positive difference is really due to the above mentioned causes was proved by determinations of the oxygen and carbon dioxide contents of the two samples and the calculation from these values of the nitrogen percentages. When correction for the volume change in

the system as given by the nitrogen values was made and consideration taken of the amount of hydrogen absorbed by the blood during the experiment, the difference in hydrogen content between the alveolar and bag samples was within the limits of error of the determination.

In order to determine the time necessary for attaining homogeneous mixture in the case of untrained subjects, a series of determinations was made on 10 medical students. The results obtained are given in table 4. In all of these experiments, the volume in the bag was 2.4 liters which appeared from the results in tables 1 to 3 to be the optimum volume for attaining homogeneous mixture in the minimum time. Samples were collected after 12 and 15 seconds' rebreathing. The subjects were instructed to rebreathe "moderately fast," the number of respirations being counted as before.

Discussion. The primary purpose of the investigation reported in this paper was to determine the time necessary for the average normal individual to attain homogeneous mixture by rebreathing a mixture of a foreign gas as used in the determination of the cardiac output. It will be seen from the above results that, in order to assume this mixture, it is necessary for most individuals to rebreathe a volume of 2.4 liters (after expiring to residual air) for at least 15 seconds before a sample taken from the bag or mouthpiece can be assumed to represent the gas in the total *lung-bag* system. This period is much longer than has usually been assumed to be necessary for attaining such equilibrium, and many results described in the literature are no doubt erroneous due to this false supposition. In some individuals (*e.g.*, A. G., table 1), mixture can be attained in 12 seconds, using 2.4 liters in the bag. In no case was more than 15 seconds required if at least 5 respirations were made and 2.4 liters were present in the bag. As regards the number of respirations necessary for attaining mixture with any given volume in the bag, it would seem, as one might indeed expect from *a priori* reasoning, that the greater the number of respirations the more efficient the mixing in a given time. Practically, however, a rate faster than about 20 to 25 respirations per minute is undesirable. The inability to properly empty the bag or expire to residual air when too fast a rate of respiration is employed, no doubt explains the inefficiency of the higher rates in attaining mixture.

As regards the volume to be placed in the bag for attaining mixture in the minimum of time, it would seem from our results that several factors are concerned in its determination. There is a volume corresponding to each individual which is optimal for obtaining mixture in the least length of time. Thus with 6 liters in the bag, mixture could not be obtained even in 20 seconds in the case of the subjects of tables 1, 2, and 3. In the case of the subjects of tables 2 and 3, 1.8 liters is definitely inferior to 2.4 liters

in its efficiency in permitting the attainment of mixture. We have, therefore, been led to adopt this last volume for the experiments on cardiac output, although a smaller volume is desirable from the standpoint of the concentration changes which occur during the rebreathing experiments.

SUMMARY

A study was made of the time necessary for attaining equilibrium between the pulmonary gases and a hydrogen-air mixture rebreathed from a rubber bag. It was found that, for the average normal subject, a period of fifteen seconds during which about 5 deep respirations are made is necessary for attaining this equilibrium when 2.4 liters are put in the bag and the subject expires to his residual air before the beginning of the experiment.

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A METHOD FOR THE DETERMINATION OF THE CIRCULATORY MINUTE VOLUME IN MAN

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The principles underlying the numerous methods which have been proposed for determining the output of the heart in man are two in number: 1, application of the Fick principle, which involves an indirect determination of the oxygen or carbon dioxide tensions in the mixed venous blood by rebreathing; and 2, the use of an indifferent foreign gas as proposed first for nitrogen by Bornstein (1910). It is the second of these principles which the present method utilizes.

A considerable number of experiments were carried out with ethylene along the same general plan as used with nitrogen (Marshall, Harrop, and Grollman, 1928) in order to avoid the errors and difficulties of the nitrogen procedure. A method utilizing ethylene was developed along these lines but proved quite cumbersome. With the experience gained from this work, we have modified the entire procedure and developed a method which is extremely simple, and which withstands all the tests of accuracy which we have applied. It is essentially a modification of the nitrous oxide method described many years ago by Krogh and Lindhard (1912). Since various criticisms of the Krogh-Lindhard method have appeared, we have attempted to test experimentally each assumption upon which the present procedure depends. Low concentrations of ethylene were used in all of the preliminary experiments which led to the development of the finally adopted method. High concentrations of ethylene and nitrous oxide have also been used, and the results obtained with the method using ethylene in low and high concentrations and nitrous oxide compared.

METHOD. The procedure which we finally adopted for determining the blood flow through the lungs and hence the output of either ventricle is as follows: A rubber bag, *D*, (fig. 1) of about 2.5 to 3.0 liters capacity is attached to a large three-way metal tap, *B*, similar to that used in respiratory work with the Douglas bag. A mouth-piece, *A*, is placed on the other end of the tap so that the subject can breathe either from the outside air, *C*, or from the bag, *D*. Between the mouth-piece and tap two small flexible

metallic tubes,[1] *F*, of 0.5 mm. bore are inserted, and the other ends arranged to fit over evacuated mercury sampling tubes, *H*. The apparatus is pictured in figure 1.

The oxygen consumption of the subject is first determined by any suitable method. We have used the Krogh (1923) recording spirometer for determining basal metabolism. While this determination is in progress, about 2.4 liters of a 4 per cent mixture of ethylene in air is introduced into the bag. The two sampling tubes are evacuated and attached to the metallic tubes, *F*. A few minutes after the oxygen consumption experiment has ended, the subject's nose is occluded with a clip, and he places his mouth over the mouth-piece. The subject now makes a quick forced expiration,

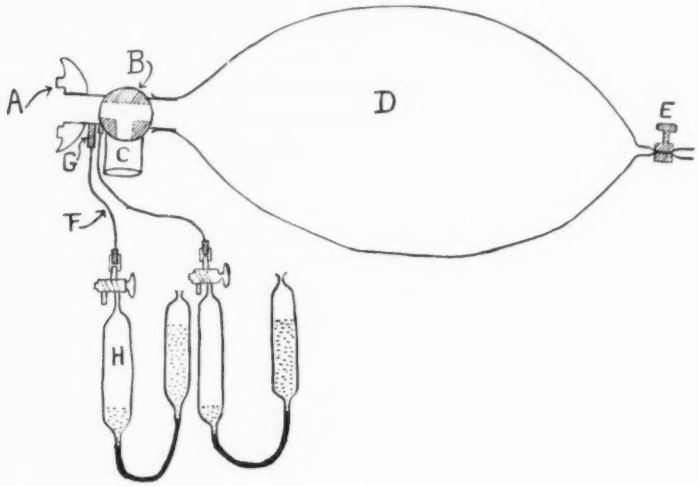

Fig. 1

the tap is turned to communicate with the bag, and the subject rebreathes from the bag, care being taken to empty the bag each time and to take at least 5 or 6 breaths in 15 seconds. The experimenter opens the tap of one of the sampling tubes 15 seconds after commencing the experiment, and the tap of the other about 12 seconds later. The experiment is now concluded by analyzing the two samples for oxygen, carbon dioxide and ethylene.

[1] These tubes may be made of ordinary capillary lead tubing or preferably of flexible copper tubing. They should be of equal length and of capillary bore to avoid the error of any dead space.

After the analyses are completed, the output of the heart may be calculated. The method of calculating the blood flow is the very simple one proposed by Lindhard (1923). The first sample is assumed to be taken at a time when complete mixture between the bag and lung air has been attained (15 seconds). The percentages of both ethylene and oxygen in the second sample are lower than in the first because the blood flowing through the lungs has absorbed a certain amount of each of these gases. If one corrects for the change in volume of the lung-bag system by means of the nitrogen percentages of the two samples, the relative amounts of oxygen and ethylene taken up by the volume of blood flowing through the lungs between the times of the first and second samples will be proportional to the difference in percentage of the oxygen content of the two samples divided by the difference in percentage of the ethylene content.

The principle of the method and calculation can be illustrated by the following:

Let V represent the volume of gas in the lung-bag system when the second sample is taken. Also let $(O_2)_I$, $(C_2H_4)^I$, $(CO_2)_I$ and $(N_2)^I$ represent the per cent composition of these gases in the first sample taken and $(O_2)_{II}$, $(C_2H_4)_{II}$, $(CO_2)_{II}$ and $(N_2)_{II}$ similarly represent the composition of these gases in the second sample. Then $\frac{(N_2)_{II}}{(N_2)_I} \cdot V$ will equal the volume of gas in the lung-bag system when the first sample was taken. $V \cdot \frac{(N_2)_{II}}{(N_2)_I} \cdot (C_2H_4)^I =$ amount of ethylene in the lung-bag system at the time when the first sample was taken, and $V \cdot (C_2H_4)_{II}$ = amount of ethylene in the same system at the time when the second sample was taken.

$$V \cdot \frac{(N_2)_{II}}{(N_2)_I} (C_2H_4)_I - V \cdot (C_2H_4)_{II} = \text{amount of ethylene absorbed during the time between the collection of samples I and II.} \quad (1)$$

If we call $(C_2H_4)_I \frac{(N_2)_{II}}{(N_2)_I}$, the corrected percentage of C_2H_4 in the first sample and represent it by $(C_2H_4)_{Icor}$, relation (1) becomes

$$V\,[(C_2H_4)_{Icor} - (C_2H_4)_{II}] \quad (2)$$

One liter of blood at body temperature absorbs 123 cubic centimeters of ethylene when the latter is at standard conditions. If the atmospheric pressure during the experiment was B, the tension of gases (exclusive of water vapor) in the lung will be B–48.3, and the amount of ethylene absorbed by one liter of blood under these conditions will be

$$123 \times \frac{B - 48.3}{760} \times \frac{(C_2H_4)_I + (C_2H_4)_{II}}{2 \times 100} \quad (3)$$

Expressing $\frac{(C_2H_4)_I + (C_2H_4)_{II}}{2}$, the average concentration in the alveoli during the period as $(C_2H_4)av$, and dividing expression (3) into (2) gives an expression (4) for the liters of blood passing through the lungs between the time of the collection of samples I and II, *i.e.*, the blood flow during this time under the conditions of the experiment:

$$\frac{V\,[(C_2H_4)_{Icor} - (C_2H_4)_{II}]}{123 \times \frac{B - 48.3}{760} \times \frac{(C_2H_4)_{av}}{100}}. \tag{4}$$

In order to derive a value for the blood flow under normal conditions, we must take into consideration the rate of absorption of oxygen during the experiment. We can derive an expression analogous to (2) for the amount of oxygen absorbed in the interval of time between the collection of samples I and II:

$$V\,[(O_2)_{Icor} - (O_2)_{II}] \tag{5}$$

This expression divided by (4) gives an expression (6) for the oxygen absorbed by 1 liter of blood during the period in question.

$$\frac{(O_2)_{II} - (O_2)_{Icor}}{(C_2H_4)_{II} - (C_2H_4)_{Icor}} \times 123 \times \frac{B - 48.3}{760} \times \frac{(C_2H_4)_{av}}{100} \tag{6}$$

All the factors involved in this last equation are known and hence the value for the oxygen utilization or oxygen absorbed by 1 liter of blood in its passage through the lungs, may be determined. It will be noticed that neither the volume of the system, V, nor the time of the collection appears in expression (6), and hence these factors need not be accurately determined in the experiment. Having determined the oxygen consumption of the individual whose blood flow is to be determined, it is only necessary to divide this quantity expressed in cubic centimeters per minute by the arterio-venous oxygen difference as calculated from (6) to obtain the blood flow or output of one ventricle per minute, under the conditions obtaining when the oxygen consumption was determined.

A typical example of an actual experiment may be quoted.

Subject E. J. P. Basal—quietly resting from 10:20 a.m. At 11:01 a.m. oxygen consumption as determined by Krogh spirometer = 224 cc. per minute. Barometer 770.7 mm. (corrected). 11:11 a.m. blood flow experiment: sample I at 15 seconds, sample II at 27 seconds.

ANALYSES	I	I (CORRECTED)	II	DIFFERENCE
C_2H_4	2.174	2.200	2.104	0.096
O_2	17.48	17.69	15.67	2.02
CO_2	5.22		6.18	
N_2	75.13		76.05	

Substituting the values found, in equation (6), gives:

$$\frac{2.02}{0.096} \times 123 \times \frac{2.139}{100} \times \frac{722.4}{760} = 52$$

Whence the circulatory minute volume is $\frac{224}{52} = 4.3$ liters.

Our procedure differs from the well-known method of Krogh and Lindhard as later modified by Lindhard (1923) principally in that the subject rebreathes continuously during the experiment and that the whole procedure is prolonged considerably beyond the time (15 seconds) recommended by Lindhard (1925) in his latest description of the method. Holding the breath and the collection of two alveolar samples is entirely avoided.

Any indirect method for determining the circulatory minute volume in man depends upon certain underlying assumptions. In the procedure which we have described the following assumptions have been made: 1, that in a lung-bag system under the conditions of our experiments mixture of the gases will result after 5 or 6 rebreathings in 15 seconds; 2, that the value 123 cc. per liter represents correctly the solubility of ethylene in different human bloods; 3, that equilibrium is attained between the ethylene in the alveolar air and the arterial blood; 4, that the time allowed for the procedure is sufficiently short to prevent any appreciable return of blood containing ethylene; 5, that the oxygen content of the mixed venous blood is constant during the procedure and exactly the same as just before the rebreathing experiment; and 6, that no appreciable error is introduced by any absorption of ethylene by the tissues of the mouth, respiratory tree, and lungs. Provided these assumptions are correct, any change in blood flow produced by the rebreathing or by any pharmacological effect of the foreign gas cannot influence the value of the minute volume as finally calculated. The main criticisms which have been brought against the Krogh-Lindhard method are 1, that incomplete mixture exists in the lungs at the time of taking the first alveolar sample; 2, that the expiration of only a portion of the air or a half expiration cannot be relied on to give a true alveolar sample, and 3, that the correction used for an increased blood flow during the experiment is not justified (Sonne, 1916, 1918; Haldane, 1922; Henderson, 1923). Krogh and Lindhard (1917) in a later communication have considered these criticisms and do not believe that they introduce an appreciable error into their method. We have avoided the second criticism and will now discuss the other criticisms in so far as they apply to our procedure as well as our justification for increasing the time of the experiment and using the rebreathing procedure along with the evidence which we have collected as to the valid-

ity of the assumptions mentioned above. Before discussing these matters, certain details of the analytical procedure used must be considered.

Analysis of ethylene. For the determination of low concentrations of ethylene in air (up to 3 per cent) the combustion method has been used. A special gas analysis apparatus (with graduations between 47 and 50 cc.) utilizing a 50 cc. sample and equipped with a Pettersson (1886) oil bubble for the control tube has been used. Since the contraction on burning is twice the volume of ethylene present, a high degree of accuracy can be attained. No rubber connections have been used, all connections being sealed glass to glass. With a long platinum spiral in the combustion chamber one minute suffices for complete combustion. Details of the combustion method, as used here, are similar to those given by M. Krogh (1914–15) for carbon monoxide analyses. An analysis requires about 5 minutes for completion. Over 3.3 per cent ethylene should be carefully avoided as a very violent explosion may occur (for explosion limits see Berl and Fischer, (1924)). The following analyses made on a large sample collected over mercury illustrate the accuracy of the method:

1.963; 1.963; 1.964; 1.962; 1.962; average 1.963 per cent ±0.001

Such accuracy is essential in using a low concentration of ethylene as the total difference in the percentages of ethylene between the two samples is only 0.050 to 0.100 per cent.

Higher concentrations of ethylene have been analyzed by absorption in a solution of 20 per cent mercuric nitrate in 2 N nitric acid (Treadwell and Tauber, 1919). Before absorption, we have found it necessary to remove the carbon dioxide as this gas is also slowly absorbed by the reagent. For our analyses we have used an apparatus with a 25 cc. burette and a Pettersson oil bubble. The absolute accuracy of an analysis is about ±0.02 per cent. The analyses take somewhat longer than the combustion method.

In both cases the oxygen and carbon dioxide have been determined on a separate 10 cc. sample by some modification of the ordinary Haldane apparatus.

Completeness of mixing in lung-bag system. When the first sample of gas is taken it is essential that the lung-bag system be a homogeneous mixture whose composition is accurately represented by this sample. Theoretically this can never be attained absolutely since exchange of gases is constantly taking place across the pulmonary epithelium. Practically, however, if the conditions are such that a homogeneous mixture would have resulted, had no gaseous exchange taken place in the lungs, no error will be involved. This is true because the absolute period of time elapsing between the two samples does not enter into the final calculation, and because the relative change in composition produced by exchange of gases

in the lungs is small compared to the total concentrations present. We have investigated the conditions necessary for mixing, and the details of this investigation are reported in a separate communication (Grollman and Marshall, 1928). It is sufficient to state here that the optimum conditions for mixing are the use of about 2.4 liters of gas in the bag, a minimum amount of air left in the lungs (residual air), complete emptying of the bag at each inspiration, and at least 5 or 6 respirations carried out in a period of 12 to 15 seconds. Individuals vary somewhat in the time and number of respirations necessary to secure homogeneous mixture. None of the individuals studied failed to obtain homogeneous mixture with 5 respirations taken in 15 seconds.

Lindhard (1925) believes that if more than 3 breaths are taken in the preliminary period of the Krogh-Lindhard method, the result may be in error. We attribute this result to incomplete mixing, but it cannot be tested with any degree of accuracy. In a few experiments we have compared the result obtained with different rates of breathing and in all cases in which mixing was complete, the results agreed within the error of the procedure. Moreover, it seems improbable that taking more than three breaths can change the composition of the returning venous blood, which is the only way in which the final result can be influenced. In a later section, the evidence for the constancy of the oxygen content of the mixed venous blood is discussed.

Solubility of ethylene in blood. The solubility of ethylene in blood must be accurately known in order to determine the blood flow by this method. Nicloux and Yovanovitch (1925) have determined the amount of this gas in the blood of dogs during anesthesia. They found that blood contained 8 to 10 vol. per cent of ethylene when the inspired air contained 65 to 77.5 per cent of the gas, and in both *in vitro* and *in vivo* experiments demonstrated that the red blood corpuscles contain a little over twice as much ethylene as the plasma.

The solubility of ethylene in human blood was determined by equilibrating at 37.5° ±0.1 about 15 cc. of freshly drawn oxalated venous blood in an 800 cc. tonometer with a mixture of 60 to 70 per cent of ethylene in air. Five cubic centimeters of this blood were extracted three or four times in the Van Slyke-Stadie apparatus, the total gas obtained from each extraction being washed with the aid of air into a sampling tube over mercury. The combined extractions and washings were transferred to the 50 cc. gas analysis apparatus and the ethylene determined by combustion. Duplicate determinations were always carried out and found to agree closely. From the analysis of the mixture to which the blood was exposed in the tonometer, the barometric pressure, and the blood gas analyses, the solubility coefficient of ethylene was calculated. The following table gives the results on the bloods of five normal subjects. α is the Bunsen absorp-

tion coefficient, i.e., the volume of gas reduced to 0° and 760 mm. which is dissolved by one volume of blood, at 37.5°, when the partial pressure of the gas is 760 mm.

TABLE 1

Solubility of ethylene in human blood

SUBJECT	α	HEMATOCRITE
E. K. M.	0.127	47.5
A. G.	0.120	47.0
T. W. B.	0.122	48.0
T.	0.123	42.5
C. L. G.	0.125	48.5
Average	0.123	46.7

We have also determined the solubility in water using the same method as for blood and found a value of 80 cc. per liter. In dog's blood the solubility is higher than in man's, being about double that for water. The average value of 123 cc. per liter for blood is much greater than the solubility in water, whereas, according to Bohr's (1905) investigations, one would expect a solubility in blood slightly less than that of water.

Equilibrium between blood and alveolar air. It is essential to demonstrate that equilibrium of ethylene is attained between alveolar air and the blood in the pulmonary capillaries. From what is known of the diffusion of carbon dioxide and oxygen from alveolar air to blood, one would expect that equilibrium would be attained with ethylene, but we have considered it worth while to test the matter by experiment. Although this could be done on man by means of arterial puncture, our experiments have been carried out on dogs and from the results obtained it seems safe to conclude that in a normal man equilibrium also occurs between alveolar air and blood.

Two experiments have been performed, the essentials of which are given below.

Experiment 1. Dog, weight 20 kilograms. Given 120 mgm. morphine and later 1 gram of sodium veronal. Tracheal tube with side opening carrying a lung catheter inserted. Arranged to breathe through valves a mixture of ethylene, oxygen, nitrogen and 5 per cent carbon dioxide. Breathing started at 0 minute. Saphenous nerve stimulated for 30 seconds to stimulate respiration. Samples of alveolar air taken by lung catheter and blood drawn from femoral artery simultaneously at various times and analyzed for ethylene.

TIME	C_2H_4 ALVEOLAR AIR	C_2H_4 BLOOD	α
	per cent	*vol. per cent*	
1′ 10″	Lost	7.10	
3′ 30″	51.9	7.18	0.147
10′ 0″	52.3	7.21	0.147

A sample of blood from the same dog equilibrated in a tonometer at 37.5°C. with 60.8 per cent ethylene gave the value of $\alpha = 0.146$.

In order to determine how quickly this equilibrium is attained the following experiment was performed.

Experiment 2. Dog, weight 24.5 kilograms. Given 180 mgm. of morphine. A large Douglas bag containing 100 liters of a mixture of ethylene, oxygen (20 per cent) and nitrogen was arranged so that it could be quickly connected to a short glass tube inserted into the trachea. In the first observation artificial forced respiration was given the animal by manual manipulation of the chest wall and bag. Fifteen seconds after commencing the breathing a sample of blood was taken from the femoral artery, and the bag removed from the tracheal tube. After breathing air, for fifteen minutes, the bag was again attached and the experiment repeated, but the increased respiration was obtained by stimulation of the central end of the cut saphenous nerve. A sample of blood was taken at 15 seconds and a second one at 3 minutes and 15 seconds.

TIME	C_2H_4 BAG	C_2H_4 BLOOD	α
	per cent	*vol. per cent*	
15″	22.93	3.48	0.159
15″	22.93	3.47	0.158
3′ 15″	22.93	3.47	0.158

A sample of blood removed from the dog at the end of the experiment was equilibrated with the mixture in the bag at 37.5° and gave a value of $\alpha = 0.157$. Another sample equilibrated with 24 per cent ethylene gave $\alpha = 0.159$, while a third sample equilibrated with 48 per cent ethylene gave $\alpha = 0.155$.

Time allowable for the rebreathing procedure. In order to obtain correct results by the procedure which we have described it is essential that the whole determination be completed before 1, any appreciable amount of the foreign gas is contained in the venous blood coming to the lungs, and 2, the oxygen content of the venous blood undergoes any appreciable change. The first of these requirements necessitates the completion of the experiment within the time of a single circulation. This time, of course, is very variable depending greatly upon the particular circuit in the body traversed by the blood, for example the marked differences which must exist in the time required for blood to traverse the coronary circuit on the one hand or to completely circulate through the foot on the other. It is essential that the time be sufficiently short so that sufficient blood does not complete its circuit and introduce an appreciable error. Blumgart and Weiss (1927) have recently found that the circulation time in the normal human subject, from an elbow vein of one arm to the brachial artery of the other arm, varies from 14 to 24 seconds. We can add at least 6 seconds

for the time necessary for the blood to go from artery to capillary and back to vein, which gives us 20 to 30 seconds as the *minimum* time of the arm circuit. It, therefore, seems unlikely that any appreciable blood could return in 25 seconds.

That the oxygen content of the mixed venous blood remains constant during the experiment is difficult to establish with certainty. This,

TABLE 2

Minute volume with different times of collection of samples

DATE	SUBJECT AND CONDITION	TIME OF COLLECTIONS OF SAMPLES AFTER BEGINNING OF REBREATHING	CIRCULATORY MINUTE VOLUME FOUND
		seconds	*liters*
May 19, 1927	T. B. (basal)	13 and 23	3.4
		15 and 30	3.4
		20 and 35	3.5
May 26, 1927	A. G. (basal)	15 and 25	4.6
		25 and 35	4.3
January 6, 1928	A. G. (non-basal)	12 and 21	4.8
		26 and 37	4.0
		40 and 56	2.4
January 9, 1928	A. G. (non-basal)	12 and 27	6.8
		24 and 37	6.4
		22 and 35	6.4
November 9, 1927	E. M. (non-basal)	14 and 26	4.6
		26 and 36	4.4
January 4, 1928	E. M. (non-basal)	15 and 30	5.1
		30 and 45	4.8
		45 and 60	3.2
January 10, 1928	E. G. (basal)	15 and 28	3.3
		20 and 35	3.1
January 13, 1928	E. P. (basal)	15 and 28	5.1
		20 and 36	4.3

however, is an underlying necessary assumption in all the methods for determining the oxygen or carbon dioxide tension of the mixed venous blood. The time allowed in various procedures of this type has been usually about 15 to 30 seconds. The main danger appears to be that the abnormal breathing during the procedure may speed up the blood flow through the lungs and from this cause accelerate the passage of blood through the

capillaries, which will necessarily change its oxygen and carbon dioxide contents if the oxygen consumption remains unchanged. Any change in the relative blood flow through areas which have markedly different oxygen utilizations would also influence to some extent the composition of the mixed venous blood returning to the right heart. Since, however, probably more than one-half the total blood of the body is on the venous side of the circulatory system, it would be some time before this effect would appear.

The most satisfactory method which we have used for estimating the magnitude of errors which might arise from these sources, and of justifying our extension of the procedure to 25 or 28 seconds, is a comparison of the results obtained when the two samples were collected at different times. The results upon several individuals would seem to prove conclusively that in 25 to 28 seconds no appreciable error is introduced from the above mentioned causes, and, indeed, show that one can in many cases prolong the time still more. The above table (table 2), gives the results.

Change in the blood flow during the experimental procedure. In the original form of the nitrous oxide method of Krogh and Lindhard (1912) a correction for the increased blood flow caused by changes in respiration during the experiment had to be made. The use of this correction has been criticized (Haldane, 1922; Henderson, 1923) on the ground that it was due to incomplete mixing. In the later method of calculation adopted by Lindhard (1923) and used by us in our procedure, such a correction is not apparent. However, calculating the oxygen consumption from the residual air of the subject and the amount of gas contained in the bag shows that in most experiments the blood flow is changed by the experimental procedure. We have proven that mixture is complete in our procedure so that the increased oxygen consumption cannot be an apparent one due to this cause. As long as the oxygen content of the mixed venous blood returning to the right heart remains constant, it is obvious that any change in blood flow cannot affect the calculation of the arterio-venous difference. It is also worth stating again that unless the oxygen and carbon dioxide contents of the mixed venous blood remain unchanged during the experiment, any form of the Fick principle, as applied to man, is invalid.

In our experiments where the residual air of the subject is known, we can calculate from the time, the oxygen difference between the two samples and the total volume of the lung-bag system, the oxygen absorption per minute during the experiment. This compared to the oxygen consumption determined with the Krogh spirometer tells us how much and in what direction the blood flow has been changed by the breathing procedure. In most cases where such calculation has been made we find an increase in blood flow, but in many instances calculation indicates that

the blood flow must have been decreased during the breathing procedure. Moreover, in duplicate estimations of the circulation carried out at the same sitting, the oxygen and ethylene differences between the two samples may be quite different while the blood flows calculated from these sets of figures agree. This can only mean that the blood flow has been affected to a different extent in the two experiments by the breathing, but that such changes do not influence the calculation of the arterio-venous oxygen difference.

In several instances where duplicate determinations of blood flow were made we have noted that in one case the experimental procedure has caused a decreased and in the other an increased blood flow, but that the value of the arterio-venous difference is the same. Two examples of markedly different changes in blood flow during the experiment may be quoted.

I. Subject's residual air = 1150 cc.; amount of gas mixture in bag = 2100 cc., both reduced to S.T.P. In the first experiment the oxygen difference of the two samples was 0.71 per cent; in the second experiment it was 1.42 per cent. Time between two samples in each experiment was 14 seconds. Calculation indicates that in first experiment oxygen absorption was 99 cc. per minute and in second 201 cc. per minute. Oxygen absorption just before rebreathing experiment was 213 cc. Therefore, the blood flow was decreased in the first to 46 per cent and in the second to 94 per cent of the normal. Arterio-venous differences for oxygen were 30 and 32 cc. per liter.

II. Subject's residual air = 1000 cc., and amount of gas in bag = 2000 cc., both reduced to S. T. P. In the first experiment the oxygen difference was 3.52 per cent, corresponding to an oxygen consumption of 105.6 cc. during 12 seconds. In a second experiment, carried out one-half hour later, the oxygen difference was 1.81 per cent, corresponding to an oxygen consumption of 54.3 cc. in 12 seconds. The oxygen consumption before the experiment was 259 cc. per minute in the first case and 257 cc. in the second. The blood flow during the rebreathing was increased 100 per cent in the first experiment, and 5 per cent in the second, over the normal. The arterio-venous oxygen differences were 63 and 60 cc. per liter of blood, respectively.

Use of higher concentration of ethylene and nitrous oxide. As pointed out by Krogh and Lindhard (1912) if the general principle of using a foreign indifferent gas for determining the blood flow through the lungs is correct, the same results should be obtained with different gases. As a matter of fact, they tried to check their nitrous oxide method by using nitrogen and hydrogen to determine the blood flow. In both cases they obtained values which were too high but explained these as being due to the fact that the tissues of the respiratory tract give off excessive amounts of nitrogen and absorb excessive amounts of hydrogen. The difficulties in the use of nitrogen have been discussed elsewhere, and hydrogen is so little soluble in blood that small errors in analyses make a large error in blood flow. To investigate the errors which may occur from the absorption of the foreign gas by the lung tissues, to rule out any disturbing pharmacological effect of the ethylene, and also to further test our method, we have attempted

to use other foreign gases. An examination of gases for their suitability indicates that few are available—ethylene, nitrous oxide, propylene, acetylene, and a few others. We have compared, in a series of individuals, the minute volumes determined by the use of low concentrations of ethylene (2 per cent), high concentrations of ethylene (15 per cent) and nitrous oxide (12 per cent).

The low concentrations of ethylene were analyzed by combustion, the high concentrations by absorption in mercuric nitrate and the nitrous oxide by combustion with hydrogen. The coefficient of solubility (α) for nitrous oxide in human blood has been taken as 0.405 (Lindhard, 1915).

TABLE 3

A comparison of the circulatory minute volumes obtained by the use of 3 different gas mixtures

SUBJECT	CIRCULATORY MINUTE VOLUME IN LITERS		
	Low C_2H_4	High C_2H_4	N_2O
E. K. M.	3.4	3.5	
E. K. M.	4.0	3.4	3.5
A. G.	3.8	3.8	3.6
G. A. H.	3.6	3.3	3.6
H. F. P.	3.3	3.0	
C. L. G.	5.0	5.0	4.5
E. J. P.	4.3	4.4	4.3
H. F. P.	4.3		4.2
B. C. M.	4.2	4.9	4.6
Average*	4.1	4.1	4.0

* Includes only those experiments in which all three methods were used.

The above table (table 3), indicates that the results of the three methods agree extremely well. All the determinations were made on subjects in the basal condition after about 1 hour's rest in a steamer chair.

Each of the three procedures used possesses certain advantages and also certain disadvantages. In the low ethylene method a gas mixture can be made up in a large Douglas bag and used for several days, as it diffuses through rubber extremely slowly. The low concentration is practically tasteless and not at all objectionable to the subject, but a special gas analysis apparatus must be used for the ethylene analyses, besides the ordinary apparatus for determining the oxygen and carbon dioxide. Despite the necessity of using two gas-analysis apparatuses the analyses can be completed very quickly and accurately. A large sample (about 80 cc.) is required for a complete analysis. In the high ethylene procedure the main objection is the disagreeable taste and odor of the mixture.

Two gas analysis apparatuses are also required here. Nitrous oxide is practically tasteless and its solubility in blood is high enough to allow sufficiently accurate analyses to be made for nitrous oxide, oxygen, and carbon dioxide in a single apparatus using a 10 cc. sample. One must take the precautions described by Lindhard (1915) but with proper care and skill a high degree of accuracy is easily obtainable.[2]

The solubility of both ethylene and nitrous oxide in blood is greater than would be expected from their solubility in water. Bohr found the solubility of chemically inert gases in blood to be 92 per cent as great as in water. The solubility of ethylene in water at body temperature is 80 cc., and in blood 123 cc. per liter instead of the calculated 74 cc. Nitrous oxide is, however, only slightly more soluble in blood than would be expected from its solubility in water (Siebeck, 1909). Since Nicloux's work proves that the increased solubility of ethylene in blood is due to the corpuscles, it is reasonable to think that marked changes in the red cell count or hemoglobin might affect the solubility. Such differences as occur in normal men do not seem to introduce any serious error (our solubility determinations on five men varied only from 120 to 127 cc. and comparison of the blood flow on seven men with ethylene and nitrous oxide gave identical results). Under changed physiological conditions in normal man or in diseased individuals where the red cell count or hemoglobin may be changed, an error may be introduced into the blood flow determination if made with ethylene; but, with nitrous oxide, the error would be much smaller and probably insignificant. Unless, therefore, the change in solubility of ethylene is corrected for in some way under these conditions it would be better to use nitrous oxide for blood flow determinations under all abnormal conditions and in all abnormal individuals.

We have used the low ethylene and the nitrous oxide procedures extensively in our work. On account of the questionable change in solubility of ethylene in blood with varying red cell count and hemoglobin, we have lately adopted the use of nitrous oxide almost entirely. Under conditions where the red cell count and hemoglobin are normal and do not change during the experiment the low ethylene procedure may possess certain advantages.

Details of the procedure. There are certain details of experimental technique involved in the determination of the cardiac output which may be briefly described.

[2] An ordinary Haldane apparatus with combustion chamber attached can be used for this analysis. We have used a modified form of this apparatus. It is advantageous to make the combustion pipette of Pyrex glass (to avoid any breakage) and to use a rather long platinum spiral (6 inches of wire, *B* and *S* 27). In place of a Kipp generator, we have used ordinary commercial electrolytic hydrogen, and removed the last traces of oxygen by absorption in pyrogallol.

The attainment of mixture as discussed above, is of paramount importance. Although 15 seconds usually suffice in normal individuals for attaining mixture, it is essential that all precautions be taken to assure this mixture. The degree of mixture attained in any case may be directly determined by the use of hydrogen (Grollman and Marshall, 1928); or duplicate determinations of the cardiac output may be made, collecting the samples at different times; *e.g.*, at 15 and 22 seconds after the beginning of the rebreathing and at 18 and 25 seconds, respectively. The results should agree if mixture is complete at 15 seconds.

TABLE 4

Rate of elimination of ethylene

Subject rebreathed an ethylene air mixture for 30 seconds. Alveolar samples were taken at various times after the rebreathing, the breath being held for 30 seconds before taking the sample, and analyzed for ethylene.

TIME AFTER REBREATHING	ETHYLENE IN ALVEOLAR AIR
minutes	*per cent*
0.0	1.880
2.5	0.11
5.0	0.03
10.0	0.00

TABLE 5

Rate of elimination of nitrous oxide

Experiment performed as in preceding table with subject rebreathing a nitrous oxide air mixture.

TIME AFTER REBREATHING	NITROUS OXIDE IN ALVEOLAR AIR
minutes	*per cent*
0.0	4.83
5.0	0.31
10.0	0.02
15.0	0.00

In doing a series of determinations on the same individual, it is necessary to wait a sufficient length of time between successive experiments, in order to allow the body to rid itself of the foreign gas (nitrous oxide or ethylene) which it has absorbed during the preceding experiment. Determination of the length of time after the completion of an experiment, during which the foreign gas could be detected in the alveolar air is shown in the above tables (tables 4 and 5).

It will be seen from the above data of tables 4 and 5 that 10 minutes suffice for the elimination of the ethylene absorbed by rebreathing it for 30 seconds while 15 minutes are necessary for the elimination of the nitrous

oxide. In carrying out successive experiments it has, therefore, been our practice to allow 15 minutes between rebreathings.

In preparing the mixtures to be rebreathed, allowance must be made for the volume of the residual air of the subject in order that the concentration of gas in the collected samples be about 1.5 to 2.5 per cent in the case of low ethylene; 10 to 18 per cent in the case of high C_2H_4 concentrations; and 10 to 15 per cent in the case of nitrous oxide mixtures. These final concentrations can be readily attained if an approximately 3.5 per cent mixture of C_2H_4 in air is used in the first case and a 20 per cent mixture of either C_2H_4 or N_2O be used in the other cases. The last two mixtures must be enriched with oxygen in order to make the oxygen content of the second sample about 15 per cent. These mixtures may be most conveniently prepared by diluting one part of a mixture of 80 per cent of the foreign gas and 20 per cent of oxygen with three parts of air.

In taking the samples, evacuated tubes are used in order to allow instantaneous collection. The sampling tubes are evacuated by means of an ordinary water pump which is connected to the reservoir-tube. Several evacuations are necessary for removing the last traces of air and care should be taken that there is no leakage of air into the sampling tubes from the time that they are evacuated until the samples are actually taken.

The exact times at which samples are taken need not be known. However, these should be known sufficiently accurately to prevent taking the first sample before mixture is attained and the second before a circuit of blood has taken place. Using nitrous oxide, it is unnecessary to prolong the procedure longer than 25 seconds. A stop-watch should be used.

Accuracy of the method. If the conditions outlined above are carefully followed, one can obtain a high degree of accuracy in the final result. Assuming that complete mixture has taken place, errors due to the rest of the procedure (analyses and determination of oxygen consumption) should not be greater than 10 per cent on a satisfactory subject. Failure to obtain complete mixture may involve an error of as much as 50 per cent. Duplicate analyses for oxygen and nitrous oxide should agree within the accuracy of the analytical method, which would involve an error in blood flow not greater than 5 per cent. The determination of the oxygen consumption on most subjects introduces only a slight error (less than 5 per cent), but an error in this determination will cause a corresponding error in the final calculation of minute volume.

Duplicate determinations made at the same sitting on a subject under constant conditions have agreed well within the above described error. The following table (table 6) illustrates the agreement of duplicate results when taken at the same sitting on a subject in the basal condition. These results include all duplicate determinations made by us during the month of January, 1928. Our later duplicate results show even better agreement than the above.

TABLE 6

Agreement of duplicate determinations of the circulatory minute volume

SUBJECT	TIME	CIRCULATORY MINUTE VOLUME	METHOD
E. M. G.	9:31	3.4	Low C_2H_4
	9:40	3.1	Low C_2H_4
B. C. M.	10:14	7.2	Low C_2H_4
	10:19	6.8	Low C_2H_4
E. M. G.	12:25	4.0	Low C_2H_4
	12:32	4.0	Low C_2H_4
E. J. P.	9:29	4.9	Low C_2H_4
	9:36	5.2	Low C_2H_4
E. K. M.	9:45	3.5	High C_2H_4
	10:00	3.5	High C_2H_4
E. K. M.	10:00	3.5	N_2O
	10:20	3.4	N_2O
A. G.	10:06	3.7	N_2O
	10:17	3.6	N_2O
C. L. G.	10:53	4.1	N_2O
	11:03	4.5	N_2O
E. J. P.	11.32	4.2	N_2O
	11:46	4.6	N_2O
H. F. P.	10:25	4.2	N_2O
	10:38	4.2	N_2O

Results on normal individuals. In the following table (table 7), are collected results which we have obtained on normal individuals. All determinations were made early in the forenoon under basal conditions. The subjects came to the laboratory without breakfast and sat reclined for an hour in a steamer chair with feet supported. All determinations were made in this posture. Most of the values are averages of duplicate determinations which agreed closely (see table 6). Together with the values for the circulatory minute volume (C. M. V.) are given certain other data and calculations which may be of interest. It is evident that with one exception all the values range from 3.0 to 5.2 liters. In the case of the two individuals on whom several determinations have been made on different days, it is

TABLE 7

Circulatory minute volume of normal individuals under basal conditions

SUBJECT	DATE	SEX	WEIGHT	HEIGHT	SURFACE AREA	PULSE	OXYGEN CONSUMPTION	CALCULATED OXYGEN CONSUMPTION*	C. M. V.	A-V DIFF.	MINUTE VOLUME		STROKE OUTPUT
											Per square meter	Per kilogram	
	1927		*kgm.*	*cm.*	*sq. m.*		*cc. per minute*		*liters*	*cc. per liter*	*liters*	*cc.*	*cc.*
T. W. B.	May 3	♂	60	177	1.77	64	200	242	3.3	60	1.9	55	52
	May 6					56	206		2.9	69	1.6	48	52
	May 13					54	205		3.1	66	1.8	52	57
	May 19					62	238		3.3	72	1.9	55	53
	May 20					56	220		3.0	73	1.7	50	54
	May 21					54	212		3.1	68	1.8	52	57
A. G.	May 14	♂	67.5	164	1.73	78	280	240	3.9	72	1.8	58	50
	May 16					70	252		3.7	68	1.7	55	53
	May 24					66	241		4.0	60	1.8	59	61
	May 25					66	277		3.6	77	1.7	53	55
	May 26						269		4.6	58	2.1	68	
	May 28						263		4.0	66	1.8	59	
	May 31						248		4.7	53	2.2	70	
	June 3						282		4.7	60	2.2	70	
	1928												
	January 23					68	263		3.8	70	1.7	56	56
	February 16					68	248		4.5	56	2.1	67	66
	1927												
E. K. M.	May 18	♂	68	181	1.86	71	244	254	4.2	58	2.3	62	59
	1928												
	January 14					67	248		3.5	71	1.9	52	52
	January 21					70	234		3.5	66	1.9	52	50
E. M. G.	January 10	♂	65	170	1.76	66	226	240	3.4	67	1.9	52	52
E. J. P.	January 30	♂	77	168	1.87	68	224	256	4.4	51	2.4	57	65
G. A. H.	January 25	♂	74.5	178	1.92	62	243	263	3.6	67	1.9	48	58
C. L. G.	January 28	♂	78	179	1.98	52	267	270	4.8	56	2.4	62	92
H. F. P.	January 27	♂	58	180	1.75	58	195	239	3.3	59	1.9	57	57
	February 1					54	197		4.2	46	2.4	72	78
B. C. M.	January 11	♀	59	166	1.66	82	213	224	7.0	31	4.2	118	85
	February 3					80	192		4.6	42	2.8	78	57
J. B.	February 20	♂	55.1	183	1.73	80	198	236	4.7	42	2.7	85	59
M. C.	February 27	♂	71.2	181	1.90	50	256	260	4.0	64	2.1	56	80
J. S. F.	March 12	♂	78	182	2.00	68	256	272	4.5	56	2.3	58	66
P. C. M.	March 14	♂	59	171	1.70	62	251	232	3.5	72	2.1	60	56
M. E. C.	March 21	♂	68.2	171	1.80	66	296	246	5.2	57	2.9	76	80
F. M. R.	March 26	♂	71.5	183	1.91	66	244	262	3.9	62	2.1	55	60
J. T. H.	April 18	♂	66.7	180	1.85	62	223	251	3.9	57	2.1	58	63

* Calculated using the Aub and DuBois standards, assuming R. Q. of 0.82 (DuBois, 1927).

† Obtained from DuBois' chart (DuBois, 1927).

evident that in one (T. W. B.) the minute volume is quite constant, while in the other (A. G.) some variation occurs (3.6 to 4.7). When determinations have been repeated at the same sitting (in the basal condition) we have never found any variation in the minute volume greater than might be ascribed to error in the method.

Comparison of results with those of other methods. It is of interest to compare the results obtained by this method with those of previous investigators. Since there is considerable evidence that in the non-basal condition and without a preliminary rest period, the circulatory minute volume may be considerably different from that obtained in the basal condition, our results are only comparable to those obtained under similar conditions. Unfortunately, very few determinations have been made in this standard condition. Moreover, results on a series of individuals, sufficiently large to make comparison worth while, are few.

Determinations using the nitrous oxide method of Krogh and Lindhard in the basal condition have been reported by Liljestrand and Stenström (1925). In ten subjects in the recumbent position they found average values ranging between 3.0 and 4.6 liters. In general, they made six determinations on each individual, three on one day and three on another. These triplicate determinations at the same sitting show maximum variations of 0.5 to 0.9 liter in most instances, but this variation may amount to as much as 2.0 liters, and in a few individuals less than 0.4 liter. From our results we may conclude that these variations on the same day cannot be due to actual changes in blood flow, but result from inaccuracies in the method employed. It is in respect to this ability to duplicate results much more closely that we consider our procedure superior as regards accuracy to that of Krogh and Lindhard.

Of the various procedures for determining the arterio-venous oxygen difference and applying the Fick principle, that of Burwell and Robinson (1924) appears to us to have been most carefully controlled and trustworthy in regard to accuracy. They report determinations on 11 individuals in the sitting position under basal conditions. These results varied between 3.5 and 6.8 liters, with only two results above 4.7 liters. Eppinger, v. Papp and Schwarz (1924), although using what appears to be a less satisfactory procedure, report results of the same order of magnitude.

We have attempted to compare our method with the Fick principle using the elimination of carbon dioxide by the procedure described by Field, Bock, Gildea and Lathrop (1924).[3] Although excellent agreement was obtained between the two methods in the case of one individual, results on two other individuals gave variable results which, in general, were

[3] We are indebted to Dr. T. W. Blake for carrying out the determinations by the carbon dioxide method, and also for assistance with some of the gas analyses in the development of our present method.

much higher than those obtained by our method. Moreover, repeated determinations by their procedure at the same sitting show much greater variation than can be accounted for by any known error in the method, and hence we consider that the average obtained from these measurements (as must be done to make a single determination) may be in error. The published results of Field and Bock (1925) in the sitting position in 10 subjects in the basal condition show values between 4.3 and 7.1 liters, but the values in the case of 8 of the 10 individuals range from 5 to 7 liters. These results are seen to be of a higher order of magnitude than those of other methods which we have recorded above as well as our own.

The results reported by the ethyl iodide method of Henderson and Haggard (1925) are in general much higher than those reported here. Recent criticism of some of the fundamental assumptions of the ethyl iodide procedure (Moore, Hamilton and Kinsman, 1926; Starr and Gamble, 1926; Barcroft, 1927; Wright and Kremer, 1927; Henderson and Haggard, 1927) make it at present difficult to attempt to harmonize the higher values obtained by this method with our own.

SUMMARY

A method is described for the determination of the circulatory minute volume in man by using a foreign gas and determining the rate of its absorption through the lungs as compared to the rate of oxygen absorption. The technique involved is simpler and the variation between duplicate determinations is much less than that obtained by the procedure of Krogh and Lindhard. The underlying assumptions of the method have been critically examined and their validity experimentally tested. Determinations of the circulatory minute volume using low concentrations of ethylene, high concentrations of ethylene, and nitrous oxide, gave values which agreed within the experimental error of the procedure. Determinations on 16 normal individuals in the sitting posture and under basal conditions, gave values ranging from 3.0 to 5.0 liters. Duplicate determinations on the same individual always agreed within 10 per cent.

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CHEMICAL STUDIES ON THE SPLEEN

II. Changes in Hemoglobin Following Removal of the Spleen

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In the previous paper of this series, Ray and Stimson (1927) presented evidence which indicated that the spleen plays a twofold rôle with respect to methemoglobin in the blood. It not only assists in the removal of this substance, but inhibits its formation. To explain these results it was necessary to assume that the spleen is capable of converting the iron existing in a ferric state in methemoglobin to the ferrous state found in hemoglobin. We, therefore, postulated a reducing action on the part of the spleen.

The question arises whether the spleen exerts a similar reducing action upon the normal blood pigment. This can be tested by studying the effect of splenectomy upon the oxygen capacity of the blood, for any chemical activity of the spleen, interacting with hemoglobin, would be manifest by a change in the properties of hemoglobin.

Doctor Stimson and the author have investigated this phase of the problem, conjointly and independently. In a preliminary report (1927) we pointed out that after removal of the dog's spleen the oxygen capacity of the blood decreased more rapidly than did the blood pigments. More recently Stimson (1927), working on rabbits, extended the investigation by showing that in these animals the non-functional portion of the pigment is an oxidation product of hemoglobin.

This report, which is a continuation of the work on dogs, analyzes the effect of splenectomy on the relation of this non-functional pigment to the total blood pigment, the functional hemoglobin, and the red cell counts.

Experimental methods. Fifteen dogs were studied in these experiments. Of these 13 were splenectomized and 2 were used as controls. Hysterectomy was the control operation. No attempt was made to accord the animals special diet or living conditions. They were fed meat scraps with bread and were given ample opportunity for exercise. In short, they were all treated as normal dogs. They remained in excellent condition,

with the exception of one which became ill with distemper and eventually died. This animal was segregated from the rest.

Once preceding and as frequently as possible after the operation[1] blood was drawn from the jugular with a syringe and placed in the tubes with oxalate. This blood was analyzed immediately in regard to its hemoglobin and total blood pigment concentration. The functional hemoglobin was estimated from the oxygen capacity as determined in the constant volume apparatus of Van Slyke and Neill. Particular care was taken to insure complete oxygenation. Not only was the blood sample subjected to a thorough preliminary oxygenation but was oxygenated a second time preceding the check determination. The limit of error allowed in the gas analysis was 0.2 vol. per cent oxygen. The total pigment was

TABLE 1

DATE	DAYS	TOTAL BLOOD PIGMENT	HEMOGLOBIN	NON-FUNCTIONAL PIGMENT*
February 3	0	19.36	19.36	0.0
February 4	0	Splenectomy (recovery uneventful)		
February 11	7	20.95	15.84	24.4
February 17	13	20.04	14.90	25.6
March 3	27	14.42	12.32	14.6
March 11	35	12.80	9.94	22.3
March 19	43	14.39	11.62	19.3
March 28	52	14.42	11.97	17.0
April 5	60	14.89	13.95	6.0
May 4	91	12.56	11.33	10.0
June 6	117	15.16	13.05	13.9
June 21	132	16.84	14.01	16.8
August 9	180	9.54	9.60	−0.67

* In per cent of total blood pigment.

determined by the method devised by Stadie (1920) for methemoglobin. This method was chosen because it offered a means of determining those substances closely allied to hemoglobin. The non-specificity of the test was an advantage as the character of the pigments was unknown It is obvious to those familiar with Stadie's method that the term total blood pigment is used in a restricted sense to mean those substances capable of being converted to cyanhemoglobin.[2]

The difference between the concentrations of total pigment and of hemo-

[1] Cf. tables and charts for actual times.

[2] Stadie's method has been criticized (Conant and Fieser, 1925) because other pigmented substances appearing in the blood may cause an error in the readings. In my experiments an excellent color match was found in all determinations, indicating a complete conversion of all pigmented substances to cyanhemoglobin.

globin, both expressed in this paper in grams per cent, gives an index of that fraction of the blood which is incapable of taking up oxygen. For want of a better name, this fraction will be referred to as the *non-functional blood pigment.* Its variations are among the most significant obtained. It is not the purpose of this paper to enter into the nature of this non-functional pigment. It may be stated here, that spectroscopic tests failed to show methemoglobin bands.

In several experiments counts of the red blood cells were also made in order to correlate changes in cell number with changes in pigments and also to serve as a morphological criterion of the condition of the red cells.

Experimental results. The course of the changes which take place in the dog's blood following removal of the spleen can be followed most clearly by presenting in detail the reactions of a typical animal. Table 1

TABLE 2

DATE	DAYS	TOTAL BLOOD PIGMENT	HEMOGLOBIN	NON-FUNCTIONAL PIGMENT	RED BLOOD CELLS 10^6
March 24	0	12.10	12.10	0.0*	468
March 24	0	Splenectomy (recovery uneventful)			
March 30	6	13.40	13.44	0.0	
April 11	20	13.84	12.12	12.4	436
April 22	31	14.49	4.97	65.0	422
April 27	36	13.10	10.36	20.8	365
May 5	44	12.50	11.16	10.7	322
June 9	79	16.34	13.02	20.3	423
June 28	98	16.33	12.68	22.3	570
August 10	142	12.50	9.80	21.6	466
August 30	163	11.30	8.50	25.5	448

* In per cent of total blood pigment.

contains the data of blood changes found in dog 2. The third column presents the variations in concentration of the total blood pigment. The concentration tends to decrease throughout the entire course of the experiment, but on the 35th day it becomes exceptionally low. After this transitory decrease to an initial minimum the total pigment increases again and later shows a second minimum on the 91st day. The final reading on the 180th day shows a marked decrease. The last column gives the values of non-functional pigment expressed in terms of per cent of total pigment incapable of taking up oxygen. The first two readings show an accumulation of this substance but on the 27th day it has been reduced to 14.6 per cent and on the 35th has increased again to 22.3 per cent. From this date it decreased, reading 6 per cent on the 60th day. This is not the end of the fluctuation, however; the non-functional pig-

ment increases again until the 132nd day and does not disappear until the 180th day.

In table 2 the changes in blood pigments are compared with variations in the number of red cells. The changes in pigment resemble those already presented in table 1. One reading, perhaps, should be explained. On the 31st day the concentration of functional hemoglobin fell to 4.97 grams per cent and the total pigment was 14.97 grams per cent. In other words, 65 per cent of the blood pigment was incapable of taking up oxygen.[3] These readings are not the result of gross experimental error; for as soon as the results of the first determination were known, a second sample was drawn and analyzed and was found to check the first. Furthermore two other dogs showed similar high figures for non-functional pigment after splenectomy.

The changes in the number of red cells are particularly interesting when considered in relation to the total pigment. The anemia occurs to about

TABLE 3

DATE	DAYS	TOTAL BLOOD PIGMENT	HEMOGLOBIN	NON-FUNCTIONAL PIGMENT	RED BLOOD CELLS 10^6
March 10	0	15.52	15.52	0.00*	580
March 10	0	Hysterectomy			
March 17	7	13.80	13.70	0.72	425
March 23	13	13.80	13.89	−0.65	457
March 28	18	13.60	13.52	0.59	570
April 11	32	12.90	12.61	2.30	454
May 7	58	13.94	13.60	2.40	628

* In per cent of total blood pigment.

the extent found by Pearce and others (1918, p. 12). The cells reach a minimum on the 44th day and return to normal on the 98th. At the end of this period the concentration of non-functional pigment remains high, indeed for 65 days after a considerable amount is still present. It must be noted here that the total pigment reaches its minimal concentration on the day of greatest anemia.

Table 3 is introduced in order that the effect of the control operation —hysterectomy—may be compared with splenectomy. The red cell count decreases slightly for several days after the operation but recovery is rapid and on the 18th day the count equals that of the control. The results also serve a second purpose, i.e., to test the reliability of the ex-

[3] It may be stated, that in spite of this low amount of functional hemoglobin, the dog in question gave no sign of respiratory distress or cyanosis. This was verified by a thorough examination with the assistance of another member of the staff.

perimental method. After the operation the concentrations of functional hemoglobin and total pigment both decrease as shown in columns 3 and 4. Both, however, decrease to about the same extent. In other words, the difference (column 5) which expresses the per cent of total pigment which is non-functional is always extremely small, never exceeding 2.5 per cent

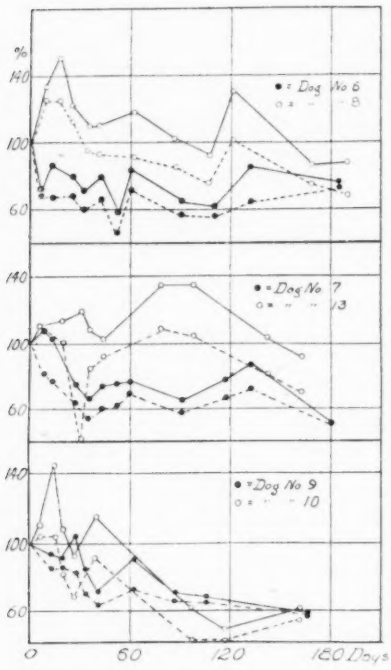

Fig. 1

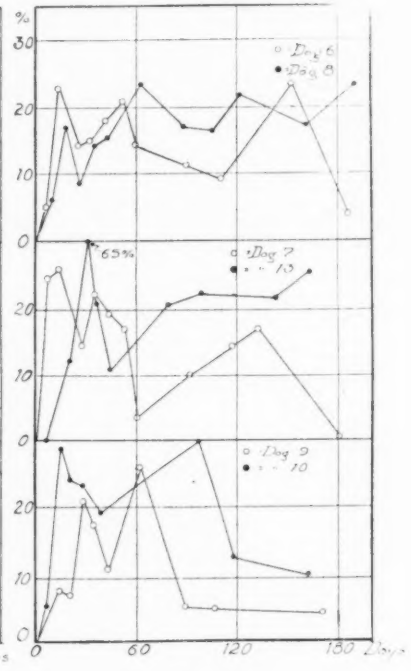

Fig. 2

Fig. 1. The changes in the hemoglobin and total pigment content of the experimental dogs following removal of the spleen. The solid lines represent the total blood pigment; the broken the hemoglobin as determined by the O_2 capacity.

Fig. 2. The formation of non-functional pigment as a result of splenectomy. The results are plotted in terms of the per cent of total pigment incapable of taking up O_2.

(0.34 gram per cent) of the total pigment. This figure is clearly within the limits of experimental error. We may conclude that an abdominal operation such as hysterectomy is not followed by a formation of non-functional blood pigment.

The plots (figs. 1 and 2) are introduced to show that the reactions described in detail are constant in the other animals studied in this series.

All the readings incorporated were made on dogs studied over the same seasonal periods thus excluding the possible influence of external environment influencing the results. In figure 1 the total pigment is plotted in solid lines and functional hemoglobin in broken lines. For the purpose of ready comparison the curves are plotted in pairs. The concentration of total pigment decreases gradually while that of hemoglobin decreases more rapidly, reaching a minimum and then tending to increase.

The distance between the two curves indicates the amount of non-functional pigment present at any time and the area bounded by the curves gives an index of the appearance and disappearance of this substance. A closer study of these curves shows clearly that it is related to the fluctuations of functional hemoglobin rather than total pigment.

In order to visualize the variations in non-functional pigment concentrations they are plotted in figure 2. While variations occur from animal to animal and in the same animal on successive days, the percentage of the total pigment which is non-functional averages about 20 per cent as a maximum figure. Such curves also bring out distinctly the uniformity with which the non-functional pigment is reduced to a minimum, approximately 30 days after splenectomy, only to increase again as the experiments are extended.

Discussion. The experiments show definitely that splenectomy is quickly followed by a marked change in character of the normal blood pigments. This manifests itself as an inability of part of the hemoglobin to combine with oxygen. This non-functional pigment at first accumulates rapidly, then diminishes, again increases, and finally reduces more gradually, the whole process taking about 6 months. This indicates that removal of the spleen is concerned with the formation of this substance but that some organ or reaction is capable of eventually compensating for the loss of the spleen.

The results have further established the fact that the formation of this non-functional pigment after splenectomy is not related to the decrease in number of red cells which is commonly regarded as an index of functional damage to the blood. Nor is there a definite relation to the total quantity of hemoglobin as determined by colorimetric methods. These observations are significant as the real test of functional disturbance is the degree to which hemoglobin will combine with oxygen. In other words anemia, in the accepted sense of a decrease in red cells, or the percental relation of the latter to the blood pigments, as given in the color indexes, may not be used to express the real functional damage which follows removal of the spleen.

The early decrease in non-functional pigment occurs parallel with the decrease in red cells, hence it might be supposed that the first step in the loss of cells following splenectomy is a loss in oxygen capacity. But this

is not true later in the reaction. At a time when the number of red corpuscles are lowest, i.e., at the time when the anemia is supposed to be most severe, the non-functional pigment decreases temporarily. During the period of recovery from the anemia non-functional pigment is present. This persists for many weeks after the cell count has returned to normal. The interaction of the spleen and the respiratory pigments of the blood seems to be distinct from that function regulating the number of red cells. Its rôle in relation to the pigments is obviously one of controlling the nature of the pigment, thus maintaining it in its active form.

SUMMARY

The red cell count, the total blood pigment, and the functional hemoglobin were determined after splenectomy of the dog. The difference between the total pigment and hemoglobin was calculated and termed the non-functional pigment. The amount of this substance at various times after the operation is a physiological index of the extent of the damage which follows removal of the spleen.

The results show it parallels neither the red cell count nor the total blood pigment, proving that these are in no sense criteria of the functional changes in the blood following removal of the spleen.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XXV. The Polycythemia of Acute Anoxemia and Its Relation to the Sympathico-Adrenal System

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The polycythemia which develops gradually during a stay of days or weeks in an atmosphere of low oxygen has received much attention because of its significance for life at high altitudes. The numerous researches directed towards explaining the phenomenon have proved that it is not due to concentration of the blood by loss of fluid (see Dalwig, Kolls and Loevenhart, 1915; and Barcroft, 1927), nor to unequal distribution of corpuscles in the body (see Izquierdo, 1922; Abderhalden and collaborators, 1926), but is a true hyperglobulia resulting from stimulation of the hematopoietic tissue by the low percentage of oxygen in the respired air (see Schneider, 1921). This slow increase in the numbers of red corpuscles is to be distinguished from the rapid increase which appears during short exposures (minutes or hours) to rarefied atmosphere. Schneider and Havens (1915), basing their judgment on the effects of abdominal massage, explained the first rise in the erythrocyte count as due to the discharge of reserve corpuscles into the general circulation that were stored in the splanchnic area. Later Schneider (1921) expressed the view that this change is "largely or wholly due to loss of fluid from the blood." The recent evidence brought forward by Barcroft and his collaborators (see Barcroft, 1926, 1927), and by Binet and co-workers (see Binet, 1927), that the spleen is a storage-place for erythrocytes, and that its contraction can greatly increase the percentage of circulating corpuscles, has given new significance to the earlier idea of Schneider and Havens. Indeed, Barcroft (1927) has himself stated that the spleen may serve as a means of rapid adaptation of the aviator to quick changes in atmospheric pressure.

The experimental evidence for this suggestion is meager. Viale and Di Leo Lira (1927), relying on a single positive experiment on a guinea pig

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and on a negative experiment on a splenectomized dog, concluded that the spleen was, in fact, an agency for adjusting the organism to low oxygen tension. They did not take the precaution, however, even in their one positive test, of excluding a possible emotional factor, which, as already shown (Izquierdo and Cannon, 1928), can by itself produce a prompt polycythemia. The observations by Binet and Williamson (1926), Binet, Cardot and Williamson (1926), Binet and Cardot (1926), and Binet and Berne (1927) that asphyxia causes a "constant, considerable, progressive and general polycythemia" were made on dogs under chloralose anesthesia. This anesthetic has been shown to be associated with a lower count of corpuscles (Izquierdo and Cannon, 1928). Chloral likewise has that effect if the spleen is intact, but not if the organ has been removed (Abderhalden and Roske, 1927). It appears, therefore, that the

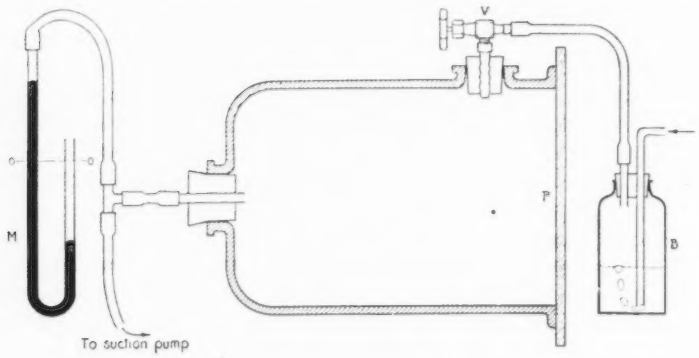

Fig. 1. Diagram of the low-tension chamber. For description see text.

anesthetic may affect the spleen in ways not yet explained. Under the circumstances Izquierdo and Cannon did not rely on the counts when chloralose was employed because the conditions were "not sufficiently free from complications." The controls cited by Binet and his collaborators were only three: two immediately after splenectomy and one while the splenic pedicle was clamped. Furthermore, in the first two the counts were already about one million higher than at the start, and the possibility of further increase even with the spleen present was not great. As controls, therefore, these tests were not satisfactory. In the third case, as in the experiments of Strohl, Binet and Fournier (1927) on guinea pigs just after laparotomy, and in those of Nitzescu and Cosma (1928) who took the blood samples from the heart of guinea pigs or from the saphenous vein or the femoral artery of dogs, the operative procedure was a possible vitiating factor.

To avoid the errors which we have indicated above—the influences of emotional excitement, anesthesia and immediate trauma—and to test the effects of low oxygen tension in healthy animals with and without the spleen, this research was undertaken.

METHOD. *The low-tension chamber.* To reduce the oxygen tension a glass chamber was employed, having a capacity of 16 liters (fig. 1). It was closed at the bottom by a greased ground-glass plate, *P*, which was clamped in place. At the top it was closed by a rubber stopper and suction tube. When in use the chamber was laid on the side. Two pumps sucked the air from the chamber, but fresh air could enter through the needle valve, *V*. By varying the opening in the valve the pressure in the chamber could be varied at will and yet a continuous flow of air (1.5 liters per minute at a mean pressure of 260 mm.) through the chamber was provided. To indicate that ventilation was continuous and regular, the air was made to bubble through thin mineral oil in the bottle, *B*. The inside air pressure was measured on the manometer, *M*, and the partial oxygen tension could then be easily calculated, with due regard to the barometer reading.

Pressures recorded in the tables were reached after 10 to 15 minutes, and thereafter were kept within variations of 2 to 3 cm. for usually 50 minutes (in a few cases for 30 minutes). The period of 50 minutes was chosen in order that the effects of any slight emotional disturbance, which might have developed when the animal was placed in the chamber, would have time to disappear (see Izquierdo and Cannon, 1928). The glass wall of the chamber permitted observations on the behavior of the animal; every ten minutes observations were made on the rate and character of the respiration. At the end of the period the pressure in the chamber was restored to normal, usually within 2 to 4 minutes.

The elimination of the emotional factor. It is clear that in the chamber just described an animal might be expected to show emotional excitement which would mask or complicate the effects of low oxygen tension. To avoid that error so far as possible the animals were used for the experiments only after becoming accustomed to the new surroundings, to the observer and to his assistants. They were kept in the laboratory at least 8 or 10 days, during which time they were repeatedly confined in the chamber until they no longer manifested any signs of excitement or any tendency to attempt escape. If later such signs appeared, the experiment was regarded as vitiated and was discontinued.

Even after the animals seemed to have become accustomed to the experimental procedures, some still had higher initial counts for a few weeks than they had later (see table 1). This may have been due to a continued slight disturbance even though no noteworthy outward indications were manifest. In other cases there were considerable variations

of the initial counts from one day to another. This was puzzling until it was discovered that the high counts were related to the time when the blood was taken—they regularly occurred if the blood was drawn in the afternoon when the animals were hungrily waiting for food. The moment the door was opened they rushed towards the attendant, mewing and holding their tails high and thereby behaving very differently from quiet, satisfied cats. The association of the high counts with this condition brings confirmation to evidence already adduced for emotional polycythemia (Izquierdo and Cannon, 1928), and indicates how sensitively the spleen reacts to emotional states.

A high initial count is unfavorable for several reasons. It may be so high that the test cannot raise it still higher; it may limit the result of

TABLE 1

Progressive fall of initial counts of erythrocyte (per cu. mm.) as cats (222, 225 and 226) became accustomed to experimental procedures

222		225		226	
Date	Counts	Date	Counts	Date	Counts
January 11	9,264,000	January 13	7,080,000	January 17	8,032,000
January 12	9,776,000	January 14	7,432,000	January 18	7,808,000
January 14	10,440,000	January 18	9,554,000	January 25	8,760,000
January 16	8,192,000	January 28	6,224,000	January 28	8,584,000
January 19	11,136,000	February 7	5,544,000	February 7	6,264,000
January 25	9,080,000	February 11	7,560,000	February 8	5,928,000
February 3	7,648,000	February 13	6,024,000	February 10	7,232,000
February 6	6,432,000	February 29	6,332,000	February 15	6,856,000
February 7	6,872,000			February 24	6,984,000
February 8	7,520,000			March 8	6,992,000
February 10	7,576,000				
February 11	7,328,000				

the test to only a minor increase; and it may actually be followed by a fall at the end of the experiment. Examples of these three types of effects may be found in the *italicised* figures of tables 4 and 7. These are not included, therefore, in the series of significant figures. They are included in the tables, however, but only to bring out strikingly the contrasting figures obtained under more favorable circumstances on the same cats.

After the effect of excitement on the initial count has been excluded, there remains the exclusion of this effect when the animals are placed in the low-tension chamber. At first, observations were made on cats merely placed in the chamber, which was kept well ventilated with air at atmospheric pressure. The results of 14 observations on 8 cats are given in

table 2. These were with one exception trained animals which showed no obvious signs of emotional disturbance while confined. Confinement for 15 minutes resulted in no change in 6 tests, and only slight increases (4.3, 4.4, 5.9 and 7.5 per cent) in four others. One cat (231), tested the second day after being brought to the laboratory, became excited in the chamber and showed an increase of 16.1 per cent. Cat 100 likewise was excited while being placed in the chamber and during the stay; the increase was 10.8 per cent; subsequent training accustomed the animal to the situation, and the test 5 days later, when the animal remained calm, resulted in no increase. In two other instances (225, 228), excitement

TABLE 2

Erythrocyte counts in normal cats confined in the well ventilated chamber during 15 minutes, for test of emotional effect

DATE	CAT NUMBER	ERYTHROCYTES PER CUBIC MILLIMETER		CHANGE
		Before	After	
				per cent
February 8	100	10,000,000	11,080,000	10.8
February 13	100	6,720,000	6,760,000	0.0
February 8	222	7,520,000	7,544,000	0.0
February 10	222	7,576,000	7,592,000	0.0
February 11	222	7,328,000	7,324,000	0.0
February 8	224	7,552,000	7,888,000	4.4
February 11	225	7,560,000	6,024,000	−17.0
February 8	226	5,928,000	6,396,000	7.5
February 10	226	7,232,000	7,096,000	0.0
February 8	228	8,792,000	9,192,000	4.3
February 13	228	7,344,000	7,640,000	0.0
February 18	228	7,728,000	7,196,000	−6.8
February 14	229	7,640,000	8,096,000	5.9
February 15	231	6,656,000	7,728,000	16.1

before the animals entered the chamber, followed by quiet while there, was attended by a reduction in the erythrocyte count.

The important question now arises, does reduction of the oxygen tension induce excitement so that decision as to the cause of a polycythemia would be difficult. Early literature (P. Bert, 1878; Richet, 1895; Carvallo, 1897) emphasized the restlessness and the discomfort of animals subjected to pressures of 400–500 mm. Hg. My own experience leads me to attribute such effects to extremely low pressures, to abrupt reduction of the pressure and to lack of familiarity of the animals with the conditions of the experiment. The importance of gradually reducing the pressure is

shown by the fact that sudden reduction to 250 or 300 mm. Hg may cause death, whereas gradual reduction to even lower pressures does not induce the deep, slow breathing which indicates respiratory distress (cf. P. Bert, 1878). In fact the animals as a rule remain quiet as the pressure falls (some of them seemed somnolent), the respiration increases in rate during the first 20 to 40 minutes and thereafter stays fairly constant (cf. tables 4 and 7), and after the test they neither hasten out of the chamber nor show resistance on entering it again. Indeed, in some instances they entered the chamber spontaneously after earlier experiences in it. Consequently, as already stated, 10 to 15 minutes were taken in bringing the pressure down, sometimes to less than a third of an atmosphere (cf. tables 4, 6 and 7). Under these circumstances no appreciable emotional disturbance—and none of the excitement resembling intoxication that has been described in man (see Haldane, Kellas and Kennaway, 1919)—but instead apathy and unsteadiness have been observed. If all these pains are taken to avoid the operation of emotional factors, the effects produced by partial deprivation of oxygen can be properly attributed to that condition alone.

Selection and condition of animals. Young vigorous cats were used for all the experiments. Some intractable animals, which did not become accustomed to the conditions of the tests during the period of preliminary training, had to be discarded. Observations were made on normal animals and on animals with the spleen denervated, with the spleen extirpated, and on one animal with denervated spleen and demedullated adrenals.

Observations in the afternoon had to be abandoned for reasons already given (see p. 148). The tests were finally made in the morning, on fasting animals. The first sample of blood was taken from a slight cut in the ear while the animal was quiet in its room. About a half-minute was required for the operation after entering the room—too short a period to permit any effect of excitement. Immediately after the animal was released from the low-tension chamber the second sample was taken.

Results. *Effects of mechanically checking respiration.* Besides the experiments on the influence of low pressure in the chamber, that have already been described, a few experiments of another kind were performed. A series of observations was made on acute asphyxia brought about by pressing for forty-five seconds between the rami of the jaw while an assistant held the cat's legs. After resumption of breathing for 2 to 4 minutes the second blood sample was taken.

The results of this procedure are given in table 3. Immediately after the asphyxia for 45 seconds no appreciable polycythemia occurred (see first test on cat 222, February 3, table 3). The erythrocyte count seemed to increase during the next 2 to 4 minutes, and in one observation made

after 10 minutes the effect may have been receding. The absence of an increase in 225 I cannot explain. This type of asphyxia, used with unanesthetized animals, is objectionable in admitting emotional factors. Furthermore, it combines lack of oxygen with excess of carbon dioxide. The experiments are serviceable only in showing the latent period of the phenomenon.

Effects of reducing the oxygen tension. The results of keeping normal cats at reduced oxygen tension equivalent to variations between 5.5 and 9 per cent of an atmosphere are presented in table 4 and figure 2A. After excluding 8 cases, which for reasons stated above (see p. 148) are attributable to excitement of the animals before entering the chamber, there remain 27 cases; in 2 the increase was 0, in 21 it was between 5.8 and 23.5

TABLE 3

Erythrocyte counts of normal cats before and after asphyxia by mechanical obstruction

DATE	CAT NUMBER	ERYTHROCYTES BEFORE (PER CUBIC MILLIMETER)	DURATION OF OBSTRUCTION	TIME BEFORE SECOND SAMPLE IS TAKEN	ERYTHROCYTES AFTER (PER CUBIC MILLIMETER)	INCREASE
			seconds	*minutes*		*per cent*
February 6	100	7,332,000	40	3	10,856,000	48 0
February 3	222	7,160,000	30	0.5	7,304,000	0 0
February 3	222	7,160,000	30	10	7,648,000	6 8
February 4	224	6,024,000	75	3	8,360,000	38 7
February 14	225	6,588,000	45	2	6,688,000	0 0
February 8	226	5,928,000	30	2	6,432,000	8 5
February 9	228	8,248,000	45	4	9,240,000	12 0
February 17	229	8,844,000	45	4	9,304,000	5 2
February 23	230	7,272,000	50	2	8,136,000	11 8

per cent, and in 4 it was more than 30 per cent. The average increase in the 21 cases of the middle group was 12.4 per cent.

Probably there is a threshold for the lowered oxygen tension which calls forth polycythemia, but the precise level of this threshold has not been determined; all tests were made at tensions which should evoke the reaction if conditions permitted its occurrence. Failure to recognize the existence of a threshold may result in employing a subthreshold anoxemia. Apparently this condition prevailed in the experiments of Nitzescu and Cosma (1928) who observed no polycythemia in a ventilated chamber kept at pressures between 300 and 350 mm. of mercury.

Granted that a threshold has been passed, is the degree of polycythemia related to the amount of reduction of the oxygen presure? Classification of the results in three groups according to the reduced pressure (320 to 280 mm. Hg,—4 cases; 280 to 240,—8 cases; and 240 to 200,—9 cases)

TABLE 4

The respiratory rate and the erythrocyte counts of normal cats subjected to low atmospheric pressure in a chamber

The respiratory rate is reported before the pressure was lowered at ten-minute intervals during the stay in the chamber and ten minutes after normal pressure was restored. The erythrocyte counts were made shortly before and immediately after the stay in the chamber.

DATE	CAT NUMBER	DURATION OF EXPERIMENT	AVERAGE CHAMBER PRESSURE	RESPIRATORY RATE (PER MINUTE)							ERYTHROCYTES (PER CUBIC MILLIMETER)		
				Before	During anoxemia					After	Before	After	Change
					10	20	30	40	50				
		minutes	*mm. Hg*										*per cent*
January 1	100	30	254	34	86	144	204			34	9,960,000	10,392,000	*4.3*
January 18	100	50	257	30	85	122		148		30	10,220,000	11,240,000	8.5
January 23	100	50	255	28	172	156			154	36	8,808,000	8,664,000	0.0
January 28	100	50	250	28	171	206	240	232	208		8,416,000	11,272,000	33.9
January 11	222	30	362								9,264,000	9,256,000	*0.0*
January 12	222	30	200								9,776,000	9,744,000	*0.0*
January 14	222	50	318								10,440,000	11,192,000	6.9
January 16	222	30	270	23	36	96	99			28	8,192,000	10,064,000	22.8
January 19	222	30	281	24		182					11,136,000	11,440,000	*0.0*
January 25	222	50	290	26	184	184	84	112	138	38	9,080,000	9,608,000	5.8
January 14	223	50	258								7,584,000	8,496,000	12.0
January 19	223	30	251	23		200					6,136,000	6,712,000	9.3
January 13	224	50	267								7,844,000	10,352,000	31.9
January 16	224	30	310	22	30		30				6,672,000	7,816,000	17.1
February 9	224	50	226	20		20	68	208	128	45	7,552,000	9,328,000	23.5
January 13	225	30	301								7,080,000	7,544,000	6.5
January 14	225	50	194	26	52	208	194		180		7,432,000	8,200,000	10.3
January 18	225	90	257	26	50	190	208				9,554,000	8,728,000	*−8.6*
January 28	225	80	220	24	75	104			86		6,224,000	6,960,000	11.8
January 17	226	50	236	23	60	78	78	120	76	24	8,032,000	8,232,000	*2.5*
January 18	226	30	247	22	36	128	126			24	7,808,000	7,472,000	0.0
January 25	226	50	256	28	50	32	22	52			8,760,000	8,584,000	*−1.8*
January 28	226	50	231	20	116	74	88	46	36	24	8,584,000	10,120,000	17.8
February 15	226	50	205	20	56	196	156	136			6,856,000	7,464,000	8.8
February 1	227	50	261	24	232	240	174	220	232	24	7,704,000	8,448,000	9.6
February 3	227	50	259	25	218	206	220	208	194		6,712,000	9,376,000	39.6
February 6	227	50	246	28		26	124	120	96		8,064,000	9,480,000	17.5
January 30	228	50	237	28	130	124		170	120		8,760,000	10,104,000	13.3
February 6	228	50	248	26	116	222	100	220	236		9,848,000	9,936,000	*0.0*
February 8	228	50	215	28	192	116	136	136	168		8,792,000	9,800,000	11.4
February 14	229	50	230	26	204	186	162	114			7,640,000	8,192,000	7.1
February 29	229	50	250	21	58	190	210	182	128		6,216,000	7,560,000	21.6
February 29	230	50	277	23	176	180	218	166	190		7,392,000	8,000,000	8.2
February 15	231	50	256	20	166	60	134	188	158		6,656,000	8,864,000	33.1
March 5	231	50	230	22	40	36	120	202	124		9,568,000	10,696,000	11.7

shows that the average increase in red corpuscles does not vary much from the average of them all, though the average in each of the last two groups is slightly higher than that of the first (see table 5).

Is the degree of polycythemia related to the respiratory changes attending low oxygen tension? In the three groups just defined, the frequency of respiration was greater the lower the pressure, but as noted above the polycythemia did not vary correspondingly. Again, in one test on the same animal (225), the respirations were rapid and in another less so, and yet there was no corresponding difference in the hyperglobulia which developed—a high count may be associated with a relatively slight rise in the respiratory rate (see cat 222, table 4) and *vice versa* (see cat 229).

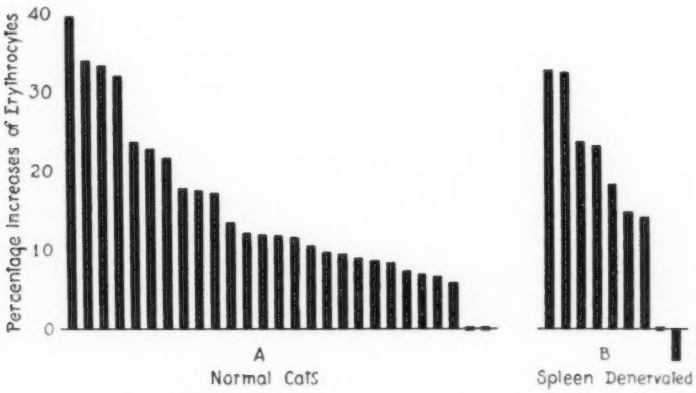

Fig. 2. Graphic record of the percentage increase of erythrocytes after exposing to low oxygen tensions normal animals (A) and animals with spleen denervated (B). (See tables 4 and 7.)

It is worthy of note that salivation was characteristic of the animals subjected to the low pressure.

Effects on splenectomized animals. As shown by experiments on cats 203 and 204 (table 6), from which the spleen was removed December 9, the characteristic polycythemia of low oxygen tension not only did not occur, but was replaced by a lower erythrocyte count than before. After a test had continued for 35 minutes on cat 204 the animal stopped breathing; air was promptly admitted to the chamber and a blood sample was taken; there was no increase, but instead the largest decrease of the series.

The foregoing observations clearly point to the spleen as the source of the anoxemic polycythemia.

Effects on animals with denervated spleen. The spleen was denervated in the manner previously described (Izquierdo and Cannon, 1928).

Observations were made on animals in which the denervation had been done 6 to 10 days previously.

With the exception of one result on cat 231 (a fall in the red count after a high initial level) the effects of low oxygen tension in these cats were as a rule greater than in normal animals (see table 7 and fig. 2B). This was

TABLE 5

Relations between progressive reduction of atmospheric pressure and (1) the average respiratory rates before, at ten-minute intervals during, and ten minutes after the stay in the low pressure chamber and (2) the percentage increases of the erythrocyte counts

RANGE OF PRESSURES	NUMBER OF OBSERVATIONS	AVERAGE RESPIRATORY RATE (PER MINUTE)							NUMBER OF OBSERVATIONS	INCREASE OF THE ERYTHROCYTE COUNT (PER CENT)	
		Before	During anoxemia					After		Individual increases	Average for each group
			10	20	30	40	50				
mm. Hg											
320–280	5	24	121	111	65	92	75	30	4	5.8; 6.5; 6.9; 17.1	9.1
280–240	13	24	114	154	165	162	176	32	8	8.2; 8.5; 9.3; 9.6; 12.0; 17.5; 21.6; 22.8	13.6
240–200	13	24	109	122	138	154	125	44	9	7.1; 8.8; 10.3; 11.4; 11.7; 11.8; 13.3; 17.8; 23.5	12.8

TABLE 6

Erythrocyte counts in splenectomized cats subjected to low atmospheric pressure in the chamber

DATE	CAT NUMBER	DURATION OF EXPERIMENT	AVERAGE CHAMBER PRESSURE	RESPIRATORY RATE (PER MINUTE)							ERYTHROCYTES (PER CUBIC MILLIMETER)		CHANGE
				Before	During anoxemia					5 to 10 minutes later	Before	After	
					10	20	30	40	50				
		minutes	*mm.* Hg										*per cent*
January 21....	203	35	270	21	124	122	Stopped breathing			82	6,872,000	5,208,000	−24.2
February 2....	203	50	308	20		96	108	84	96	36	7,544,000	6,952,000	−7.8
January 21....	204	50	267	24		132		128	126		8,648,000	7,992,000	−7.5
February 4....	204	50	247	24		162	156		116		7,632,000	7,776,000	1.8

a surprising result, for excitement was largely ineffective under such conditions (see Izquierdo and Cannon, 1928); it was invariably ineffective after short periods of excitement,—the positive cases (increases of 5 to 20 per cent) occurred only after excitement for periods lasting from 5 to 10 minutes. The stimulus of anoxemia was applied for periods two or three times as long. The explanation offered for the irregular results of

excitement, after denervation of the spleen, was that the organ failed to concentrate consistently the blood in its pulp, and that the excitement at different times varied sufficiently to produce different effects. It is noteworthy that injections of adrenalin yielded more regular results. These facts taken together suggest perhaps that the data presented in table 7 can be explained by a more prolonged discharge and action of adrenin than was obtained in the experiments on the influence of excitement. A number of considerations support this suggestion:

A. The animals in the chamber display numerous signs of sympathetic stimulation. Salivation occurs, an effect of sympathetic or sympathico-

TABLE 7

The respiratory rate and the erythrocyte counts of cats with denervated spleen that were subjected to low atmospheric pressure in a chamber

The respiratory rate is reported before the pressure was lowered, and at 10 minute intervals during the stay in the chamber, and 10 minutes after normal pressure was restored. The erythrocyte counts were made shortly before and immediately after the stay in the chamber.

DATE	CAT NUMBER	DURATION OF EXPERIMENT	AVERAGE CHAMBER PRESSURE	RESPIRATORY RATE (PER MINUTE)							ERYTHROCYTES (PER CUBIC MILLIMETERS)		
				Before	During anoxemia					After	Before	After	Change
					10	20	30	40	50				
		minutes	*mm. Hg*										*per cent*
March 15	225	50	250	23	26	69	64	50	44	22	7,120,000	7,592,000	14.8
March 16	225	50	257	23	34	54	42	50	38	24	5,448,000	7,232,000	32.5
March 15	226	50	250	22	26	36	35	37	36		7,480,000	8,532,000	14.0
March 16	226	50	260	24	52	32	39	39	30		6,024,000	7,440,000	23.5
March 18	229	50	240	23	32	100	186	132	80	28	5,688,000	7,000,000	23.0
March 18	229	50	250	24	28	116	52	38	34		5,656,000	7,480,000	32.2
March 1	230	50	247	24		72	140	136			7,344,000	8,680,000	18.1
March 17	231	50	248	24	58	60	60	58	58		8,384,000	8,088,000	−4.0
March 18	231	50	238	23	36	122	130	162	134	20	7,400,000	7,672,000	0.0

adrenal action in the cat (Luchsinger, 1877; Langley, 1901; Florovsky, 1917). The hairs of the back and tail are erect. The pupil shows an oscillating enlargement. Furthermore, anoxemia causes secretion of adrenin (Kellaway, 1919; Houssay and Mollinelli, 1926), even though the nerve supply to the glands has been removed (Zwemer and Newton, 1928). Moreover, removal of the medulla of each adrenal gland in cat 225, which had shown increases of 14.8 and 32.5 per cent after denervation of the spleen, resulted in *decreases* thereafter in the erythrocyte count (April 12; initial count 7,656,000, after 50 minutes at 228 mm. Hg, 6,600,000: April 18; initial count 8,200,000, after 50 minutes at 256 mm. Hg,

7,392,000). These observations not only show that conditions are present for medulliadrenal secretion, but they indicate that it is important for the polycythemia which appears after splenic denervation.

B. There is evidence that recently denervated spleens are lacking in tone. Cats 226, 229, 230 and 231 were killed by asphyxiation in the chamber. The spleen in each instance was promptly removed and weighed. The spleens of the first three animals weighed respectively 25, 27.5 and 29 g.; that of cat 231 had the usual *post mortem* weight of the cat spleen—7 g. If the very large spleens of three of the animals may be taken as typical (that of 231 being exceptional, perhaps because of a longer period after the denervation), and if the blood in these spleens is concentrated, contraction of a recent denervated and somewhat dilated spleen might be expected to yield the unusually large increases reported in table 7.

C. Organs deprived of their sympathetic nerve supply have increased sensitiveness to adrenin. This has been proved true of the smooth muscle of the cat's iris (Lewandowsky, 1899; Elliott, 1904), of the nictitating membrane (Auer and Meltzer, 1908), the *arrectores pilorum* of the head (Langley, 1901), the vessels of the rabbit's ear (Meltzer and Meltzer, 1903, 1904; Lichtwitz and Hirsch, 1910), and of the frog's leg (Pearce, 1913; Okuyama, 1926), the gravid uterus (Ogata, 1921), and of pilomotor and vasomotor reactions in man after peripheral nerve section (Lévai, 1926). With regard to the spleen Hou (1927) observed that the denervated portion of that organ contracts more intensely than the normally innervated portion during exercise and inferred that the effect was due to sensitization to adrenin.

It is possible that all three of the conditions mentioned above are important in producing the polycythemia which occurs after anoxemia for 50 minutes in an animal with denervated spleen.

Effects of a mixture of 20 per cent carbon dioxide and normal air. This mixture results in a reduction of the oxygen percentage to about 17 per cent. Furthermore, the increased tension of carbon dioxide in the blood would reduce the saturation of the red blood corpuscles with oxygen. Such information as is available, however, indicates that the oxygen delivery to the tissues is not seriously diminished by this combination of effects. The results obtained when normal cats are placed in an atmosphere having this high content of carbon dioxide support this inference (see table 8). Two observations in table 8 must be excluded from consideration because of the presence of the emotional factor (cat 100, and cat 226 on March 8). The results of the remaining 9 observations contrast strikingly with those obtained by producing anoxemia; 5 of the 9 were negative, 2 were close to the limit of accuracy of the method, and an increase of 11.1 per cent was maximal in the remaining 2 cases. These

negative effects of 20 per cent carbon dioxide accord with those of Barcroft and Stephens (1927) who found no noteworthy contraction of the spleen on placing dogs in air containing 5 per cent of the gas: and also with those of Kellaway (1919) who discovered that lack of oxygen rather than increase of carbon dioxide was the effective stimulus for sympathico-adrenal activity.

The breathing of excess of carbon dioxide in the experiments just cited was not attended by noteworthy salivation. The respiration was increased in amplitude rather than in rate which was the characteristic reaction to anoxemia (Meakins, Haldane and Priestley, 1919; and Haldane, 1911).

TABLE 8

Respiration rates and erythrocyte counts of normal cats that remained ten minutes in a chamber ventilated at about atmospheric pressure with air containing 20 per cent carbon dioxide

DATE	CAT NUMBER	DURATION OF EXPERIMENT	RESPIRATORY RATE (PER MINUTE)						ERYTHROCYTES (PER CUBIC MILLIMETER)		
			Before	During					Before	After	Change
				2	4	6	8	10			
		minutes									*per cent*
February 28	100	6	18	24	26	40		39	10,248,000	11,168,000	*8.9*
February 29	225	6	24	36	40		42	48	6,332,000	7,040,000	11.1
February 24	226	6	24	36		43	46	52	6,984,000	6,872,000	0.0
March 8	226	10	20	20	23	30	32	36	6,992,000	8,592,000	*22.8*
February 28	228	6	25	44	44	44			8,840,000	9,608,000	8.6
March 2	229	6	16	22	26	28	38	51	6,624,000	6,632,000	0.0
March 5	229	6	22		28	30			7,544,000	7,872,000	4.3
February 28	230	6	18	26	20	24	36	70	7,896,000	9,016,000	0.0
March 6	231	6	21	22	24	26	40		9,736,000	9,552,000	0.0
March 8	231	10	20	19	24	24	26	30	9,056,000	8,704,000	0.0
February 28	232	6	24	24	38	32	60	74	6,232,000	9,048,000	4.5

SUMMARY AND CONCLUSIONS

Brief asphyxia by mechanical obstruction of the air passage in normal animals is followed promptly by polycythemia, but the effect is probably largely due to the attendant excitement of the animal.

Low oxygen tensions (from 5.5 to 9 per cent) do not provoke excitement if they are slowly established and if the animals have become accustomed to all the circumstances of the experiment.

Such low oxygen tensions induce in cats which remain quiet and serene rapid and shallow respiration, salivation, erection of hair, and polycythemia.

Removal of the spleen is followed by failure of the anoxemic polycythemia.

Anoxemic polycythemia evoked about a week after denervation of the spleen may be greater than in the normal animal. Reasons are given for inferring that this effect may be due to extra adrenin acting on the toneless spleen; it disappears if the adrenal glands are demedullated.

Increase of carbon dioxide in the respired air (even as high as 20 per cent) has little or no effect in producing polycythemia.

Normally a rapidly developing anoxemia would be accompanied by emotional excitement, and the two conditions would coöperate to call forth extra red corpuscles from storage in the spleen. Nervous and humoral factors would both operate in producing this effect. These results illustrate again the emergency functions of the sympathico-adrenal system.

I take pleasure in expressing to Prof. W. B. Cannon my deep gratitude for an enjoyable and profitable year spent in his laboratory.

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SOME FURTHER FACTORS INFLUENCING THE NORMAL ISOMETRIC PERIOD OF THE HUMAN HEART

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As stated in a previous preliminary paper (1), there has been great diversity among investigators regarding the normal length of the isometric contraction period of the human heart. The purpose of this communication is to examine some of the factors which normally modify it.

The period itself is defined, following Robinson and Draper (2) as "the time during which the pressure in the ventricle is rising to overcome the arterial pressure."

The method of measurement is that suggested by Wiggers and Clough (3) which is based upon the observation that when simultaneous optical records of the heart sounds and the carotid pulse tracing are recorded, the second sound is synchronous with the bottom of the carotid incisura and therefore these points give a method for estimating the time interval between the first sound and the primary upstroke of the carotid—the boundaries of the period in question.

In the preliminary paper above mentioned, it was pointed out that when a series of records of the isometric periods of men in the standing position were arranged in descending order of their respective cycle lengths, the isometric periods tended to vary inversely with the cycle length. In this communication it is proposed to extend the scope of the investigation to include data obtained from women subjects, as previous work on the length of the systole had shown a marked sex difference in the behavior of the heart. The conclusions in this paper are drawn from an investigation of records from fifty-nine men and sixty-five women in the standing, sitting and recumbent positions, respectively, but because of uncertainties in reading of some curves, the final data were taken from the records of forty men standing, thirty-six men sitting, thirty-six men recumbent, fifty women standing, fifty women sitting, and thirty-four women recumbent.

At the time these records were taken the blood pressure of each individual was taken in each of the three positions, with the idea that some relationship between the diastolic pressure and the isometric period might be demonstrated. However, it was found that the recorded differences in

pressure as produced by changes in position were so slight that they were at any time less than the normal error of reading, i.e., less than 10 mm. Hg. and therefore could not be used as a basis of conclusions. Also, no relationship could be determined between the ages of the subjects examined (17 to 55 years) and their respective isometric periods.

It has been previously shown (4) that there is a definite relationship between the length of the systole and that of the cycle and it was expected that a somewhat similar relationship might exist between the cycle and the isometric period. Hence the data obtained from the subjects as above described were arranged in descending order of cycle length and the isometric periods were plotted as ordinates against cycle lengths as abscissae (see fig. 1). The resulting curve shows a decided tendency to increase with a decrease in the length of the cycles, while the diastoles, plotted similarly, showed a decided shortening with decreasing cycle lengths.

To determine the possible effects of position on the isometric periods of both men and women, the records for the respective positions were plotted as shown in figure 2, in which the average isometric periods for men and women in each of the positions are plotted as ordinates against cycle lengths as abscissae, in descending order of length. From these figures it will be seen that there is a decided tendency in the standing position for the isometric period to increase with decreasing cycle length, this tendency being less marked in the recumbent position and absent in the sitting position.

From our previous work on the length of systole (5) it was expected that a distinct sex-difference in the length of the isometric contraction period would be observed, but an examination of the curves of figure 2 shows that there is practically no sex-difference to be observed, so far as these results are concerned.

In explanation of the fact that the isometric period not only does not shorten with lessening cycle lengths, but that, in the standing position particularly, it actually increases with quickening heart rates, it is suggested that the length of the isometric period is influenced largely by the amount of blood in the ventricle at the beginning of the period, i.e., at the end of diastole. In the standing position and at fast rates, owing to the effect of gravity to retard the flow of blood into the heart, the amount of blood in the ventricle at the end of diastole is relatively small and the pressure correspondingly low. The heart therefore will have to do relatively more work to raise the pressure sufficiently to overcome the diastolic load and open the aortic valve. In long cycle lengths in the standing position, and to a certain degree in all cycle lengths in the other positions, either there is time enough for blood to accumulate in the veno-auricular reservoir, or its return is not hindered by gravity. Consequently, with a relatively larger amount of blood in the ventricle, the difference between the intra-

Fig. I

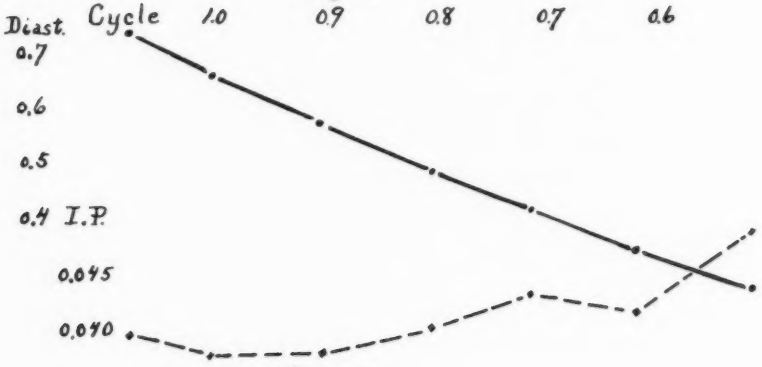

Fig. II

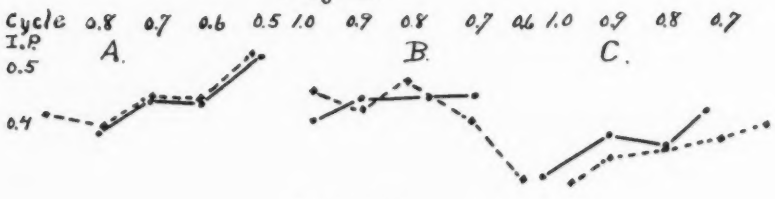

Fig. III

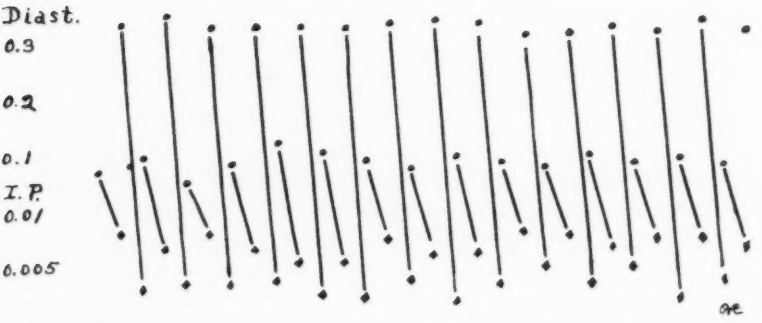

Fig. 1. Relation of isometric period to diastole length. Abscissae, cycle lengths; ordinates: upper curve, diastole lengths; lower curve, isometric periods.

Fig. 2. Effect of position on isometric period. Abscissae, cycle lengths; ordinates, length of isometric periods. Solid lines, men; broken lines, women. Curve A, standing position; B, sitting; C, recumbent.

Fig. 3. Relation of length of diastole to succeeding isometric period in curve 169, showing "coupled beats." Upper dots, successive diastole lengths; lower dots, isometric periods. Each diastole is connected with its following isometric period.

ventricular pressure and the diastolic arterial pressure tends to become less than in the previous case, and the isometric period tends to be shorter.

The above deductions all point to the length of the diastole as the predominating factor in determining the length of the isometric period. An examination of figure 3, in which the lengths of the successive diastoles and of the isometric periods of an individual with "coupled beats" are plotted in the order of their occurrence, will show this relation very plainly. It will be seen that each long diastole is followed by a relatively short isometric period, and *vice versa*.

In conclusion, it is noted that the average isometric period of the human heart varies between 0.030 second and 0.060 second, with an average of 0.0417 second. This figure is apparently modified but little by age, sex or blood pressure, but is altered by heart rate, particularly in the standing position, because its length is determined largely by the length of the preceding diastole, this influence being due to the amount of blood and therefore the pressure in the ventricle at the close of diastole.

Having determined the normal length of the period, it is proposed to continue the investigation by observing the effects of pathological circulatory conditions on the period in question.

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REGULATION OF RESPIRATION[1]

XIX. Central and Peripheral Action of Sodium Cyanide on Respiratory Movements

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It is generally assumed that somewhere in the brain stem a grouping of nerve cells designated as the respiratory center controls pulmonary ventilation by rhythmic discharge and stimulation of motor nerve cells. Though the exact mechanism of this control is unknown, the activity of the center appears to be influenced in two ways,—by impulses reaching it from the periphery via the afferent nerve fibers of the body, or by chemical changes occurring in the center. The relative significance of these factors is not always easily evaluated. Prompt apnea elicited by central stimulation of afferent fibers constitutes a demonstration of peripheral nervous control, but apnea following excessive ventilation is interpretable in several ways. It may be due to excessive mechanical stimulation of afferent nerve fibers, or it may be due to chemical influences exerted directly on the respiratory center, or possibly, to chemical influences on the peripheral afferent mechanism.

That chemical changes occurring in the blood modify ventilation by direct action on the respiratory centre was indicated by the crossed circulation experiments of Fredericq, 1901. That the conclusions of these important experiments were carried too far has recently been demonstrated by Heymans and Heymans, 1927, who have definitely shown the possibility of peripheral humoral control as well as central humoral control. In crossed circulation experiments in which the head and trunk of an animal are humorally separated and connected only by the vagus nerves it has been found that administration of carbon dioxide to the isolated trunk augments respiratory movements of the isolated head.

We are thus confronted with the question of the relative significance of central and peripheral humoral control. That central chemical control is significant, Heymans and Heymans have satisfactorily demonstrated for

[1] Reported before the Federation of American Societies for Experimental Biology. Proceedings of the American Physiological Society, This Journal, 1928, lxxxv, 373.

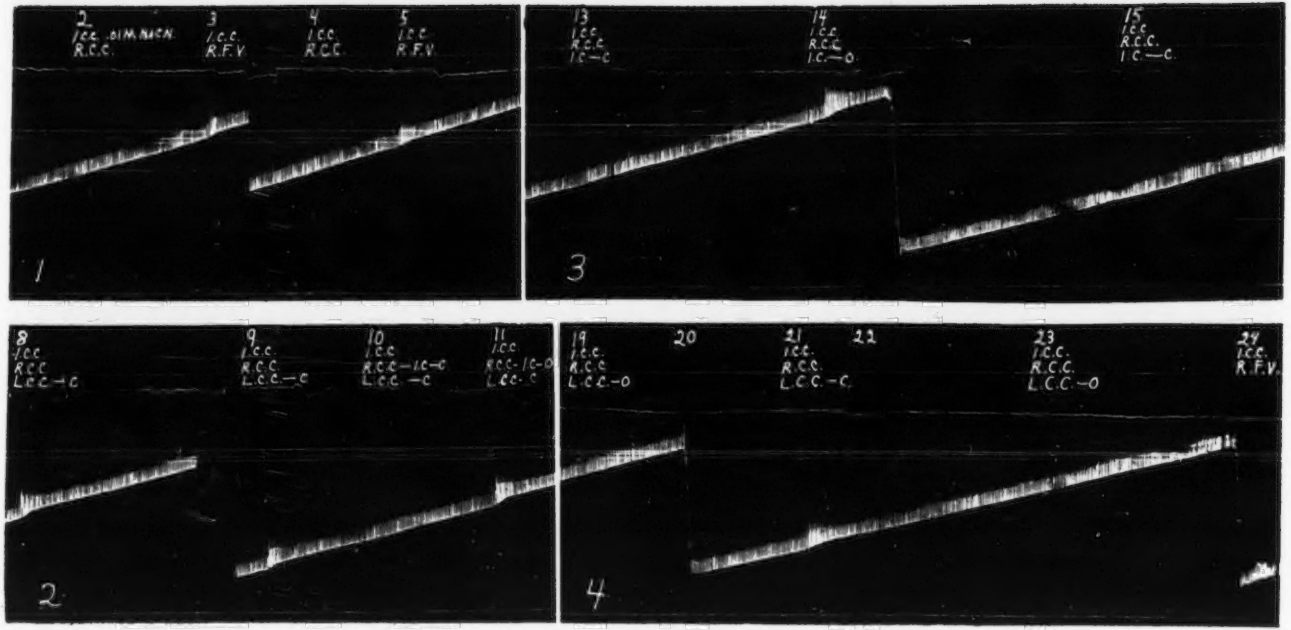
themselves by severing afferent fibers in the isolated head. Such preparations react strongly to chemical changes in the blood. On the other hand the sensitivity of the isolated head (connected with the trunk by the vagus nerves) to chemical changes in the trunk strongly suggests to them the possibility of a normally functioning peripheral humoral control.

Though we (Hertzman and Gesell, 1927) have definitely confirmed peripheral chemical stimulation of the vagal endings with the administration of carbon dioxide to the isolated trunk our results were not as constant nor as striking as were those of Heymans and Heymans. This is possibly due to peculiar differences in conditions which we have provided and we are, therefore, not inclined to discount the significance of peripheral chemical action, but rather, to investigate the question further.

In the crossed circulation experiments previously reported we have shown that administration of sodium cyanide or of a gaseous mixture low in oxygen to the isolated trunk was relatively ineffective in stimulating respiratory movements of the isolated head. Only with massive injections of sodium cyanide was stimulation observed. These results are in agreement with those of Heymans. Since these findings cannot be said to definitely exclude a peripheral chemical stimulation of the vagus ending with cyanide in the intact animal it seemed worthy of trial to study the action of cyanide from a slightly different angle—to test the relative effectiveness of intra-arterial and intravenous administration.

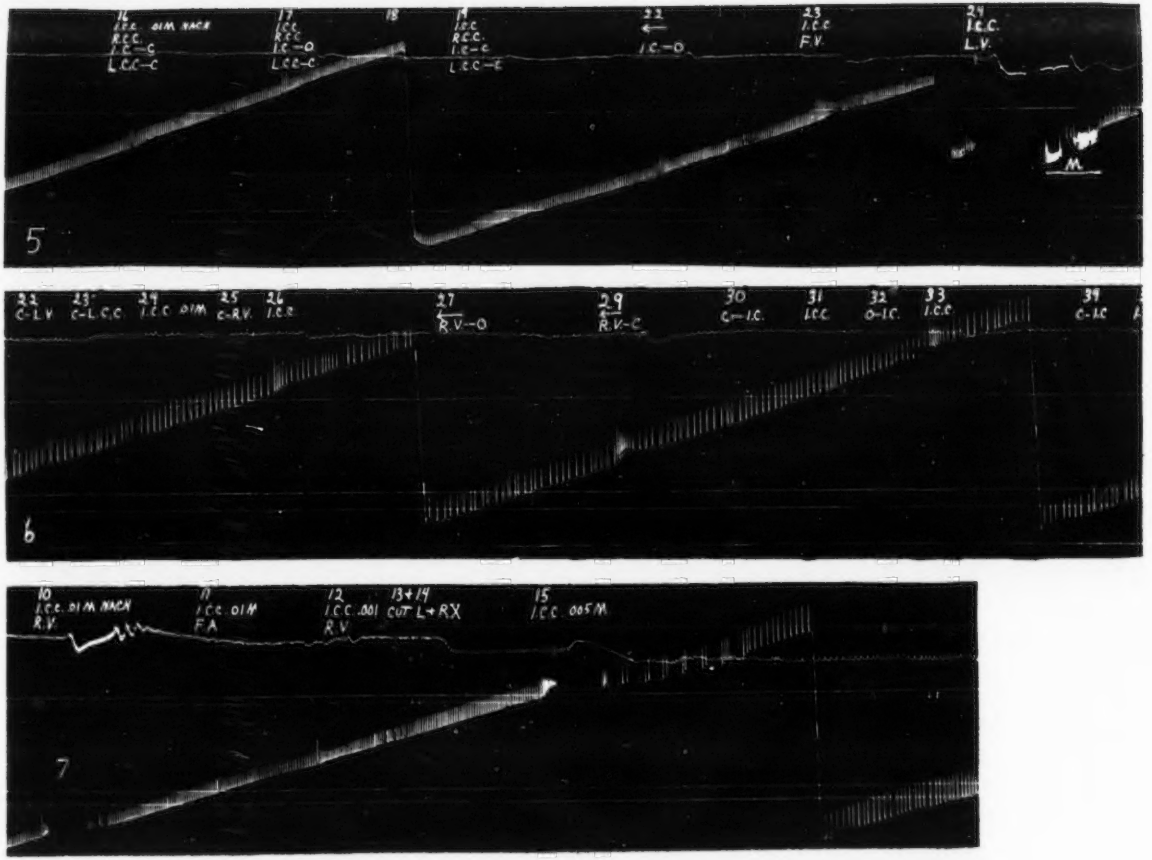
In a few trial experiments done in conjunction with our crossed circulation experiments (Hertzman and Gesell) peripheral chemical stimulation in the femoral region was tested. Sodium cyanide sufficient to produce intense stimulation when injected intravenously was injected into the femoral artery. It was assumed that if peripheral chemical stimulation occurred it would appear promptly and before the cyanide was carried to the central nervous system, but no stimulation whatever was noticed. The injection was increased with the same results. Apparently the cyanide was absorbed by the leg and never reached the general circulation.

In crossed circulation experiments in which the isolated head received all of its blood through the common carotids which are connected with the common carotids of the donor, direct injection into the carotid artery produced intense stimulation of the respiratory movements of the isolated head but no effect whatever was noted in the respiratory movements of the donor. The conclusions from these observations are: that respiratory movements may be stimulated independent of the vagus endings, that the respiratory center may be directly stimulated by the action of cyanide, and that the brain possesses the power of fixing cyanide similar to that of muscle. These observations by themselves, however, do not necessarily prove that the excitation of the isolated head is central, as is generally assumed, for it must be remembered that the head and upper respiratory tract are well


2
1 c.c. SIM. MICH.
R.C.C.
3
1 c.c.
R.F.V.
4
1 c.c.
R.C.C.
5
1 c.c.
R.F.V.
1
13
1 c.c.
R.C.C.
1 c—c
14
1 c.c.
R.C.C.
1 c.—o.
15
1 c.c.
R.C.C.
1 c—c.
3
8
1 c.c.
R.C.C.
L.C.C.—c
9
1 c.c.
R.C.C.
L.C.C.—c
10
1 c.c.
R.C.C.—1 c—c
L.C.C.—c
11
1 c.c.
R.C.C.—1 c—o
L.C.C.—c
2
19
1 c.c.
R.C.C.
L.C.C.—o
20
21
1 c.c.
R.C.C.
L.C.C.—c
22
23
1 c.c.
R.C.C.
L.C.C.—o
24
1 c.c.
R.F.V.
4

Figs. 1 to 4


16
1 c.c. SIM. MICH.
R.C.C.
1 c—c
17
1 c.c.
R.C.C.
1 c—o
18
19
1 c.c.
R.C.C.
1 c—c
22
1 c—o.
23
1 c.c.
F.V.
24
1 c.c.
L.V.


16 I.C.C. .01M NACN R.C.C. I.C.—C L.C.C—C
17 I.C.C. R.C.C. I.C—O L.C.C—C
18
19 I.C.C. R.C.C. I.C—C L.C.C—C
22 ← I.C—O
23 I.C.C F.V.
24 I.C.C. L.V.
5
22 C-L.V
23 C-L.C.C.
24 I.C.C .01M
25 C-R.V.
26 I.C.C.
27 R.V—O
29 R.V—C
30 C—I.C.
31 I.C.C.
32 O-I.C.
33 I.C.C.
39 C-I.C
6
10 I.C.C. .01M NACN R.V.
11 I.C.C. .01M F.A.
12 I.C.C .001 CUT L+RX R.V.
13+14
15 I.C.C .005M
7

Figs. 5 to 7

provided with afferent nerve endings which normally exert a powerful influence on respiratory movements.

The probability of such peripheral chemical action of cyanide is considered in this paper. So far as I am aware sodium cyanide has always been administered intravenously, thus reaching all parts of the body at approximately the same time. Knowing that the tissues effectively absorb small quantities of cyanide administered intra-arterially, cyanide administered by the carotid artery should be physiologically active in the head region only. The action on the endings of the vagus nerves can thus be easily avoided in the intact animal. When such injections were made into the right common carotid (1 cc. 01 M sodium cyanide with a very fine needle while the blood is flowing freely through the carotid) contrary to expectation no stimulation whatever was produced. The same amount injected into the femoral vein, however, invariably excited respiration, though the cyanide by such administration must have reached the central nervous system in greater dilution. These results are seen in observations *2* and *3* of figure 1 in which the arterial injection was made in the right common carotid. These are repeated in observations *4* and *5*. It must be admitted that momentarily these findings suggested that the vagus endings are effectually stimulated in the intact animal when in good condition, though such action be missing in the crossed circulation experiments in which the condition of the trunk is markedly impaired. But when the same injection was made in the right common carotid while the left common carotid was occluded stimulation of the respiratory movements occurred as is seen in observations *8* and *9* of figure 2 and *21* of figure 4. In figure 4 alternate injections were made with the left common carotid open and closed. At *19* and *23* when the artery was open, stimulation from injection was missing. It was present when the artery was occluded and when injection was made into the femoral vein. This suggested that occlusion of one common carotid artery shunted the flow of the other common carotid to lower levels in the brain stem where cyanide stimulates respiratory movements. This suggestion was substantiated in observation *10* in which cyanide was injected into the right common carotid while the right internal carotid and left common carotid were occluded. Stimulation failed to appear presumably due to the fact that cyanide could no longer reach the circle of Willis in abundance, for the amount passing through the anastomosis with the external carotid via the ophthalmic artery would be small. The cyanide is thus shunted through the external carotid into relation with the afferent peripheral endings without action. De-occlusion of the internal carotid and shunting of the cyanide to the centers at 11 again provoked augmented ventilation.

Further results appear in figure 5 obtained from another dog. At *16* and

19 1 cc. 0 01 M sodium cyanide was injected into the right common carotid the right internal carotid and left common carotid being occluded. At *17* and *22* similar injections were made into the right common carotid with the right internal carotid de-occluded. At *23* and *24* equal injections were administered into the femoral vein and left vertebral artery respectively. At *17* and *22* distinct augmentation of respiratory movements occurred while at *16* and *19* there appears to be only the slightest increase. Whether this increase is attributable to peripheral stimulation is questionable for the anastomosis of the external carotid with the circle of Willis via the ophthalmic artery may carry sufficient cyanide to the respiratory center, for it will be noted that the left common carotid was clamped which would tend to shunt the blood from the upper portion of the circle of Willis to lower levels. At *23* the usual stimulating effects of intravenous injection occurred. Injection into the vertebral artery produced profound effects,—a momentary augmentation of ventilation followed by severe depression. The animal was resuscitated by thoracic massage (M). These severe effects illustrate the difficulty of adjusting vertebral injections to the point of stimulation only. Unless very weak solutions of cyanide are used depression follows rapidly on excitation.

Figure 6 is taken from another experiment in which respiratory movements were considerably depressed by morphine. At *22* and *23* the left vertebral and left common carotid respectively were occluded and at *24* 1 cc. 0.01 M sodium cyanide was injected into the right common carotid without apparent effect. At *25* the right vertebral artery was occluded. Injection of 1 cc. of sodium cyanide into the right common carotid now augmented ventilation. A second injection at *27* following de-occlusion of the right vertebral was without effect, but became effective again at *29* on occlusion of the right vertebral. At *30* the right internal carotid was occluded. Injection of cyanide at *31* was ineffective though both vertebrals and the left common carotid were occluded. Obviously the flow of cyanide through the internal carotid provides the stimulating factor as illustrated again by injection at *33* where the right internal carotid is de-occluded.

In figure 7, 1 cc. of 0.01 M sodium cyanide was injected into the right vertebral artery with the usual result of initial excitation and subsequent depression followed by recovery. At *11*, a similar injection was administered into the femoral artery. At *12*, 1 cc. of 0.001 M solution was injected into the right vertebral artery. At *13* and *14*, the left and right vagus nerves were cut; and at *15*, 1 cc. of a 0.005 M sodium cyanide solution was injected into the right vertebral artery. The stimulating effects were as great as with the vagus nerves intact.

SUMMARY AND CONCLUSIONS

The relative effects of central and peripheral chemical stimulation of respiratory movements by sodium cyanide were studied by means of intra-arterial and intravenous injection.

Evidence is presented indicating that cyanide injected intra-arterially in small amounts is fixed locally by the tissues and does not reach the general circulation. Chemical stimulation should, therefore, be limited to the local distribution of the artery involved.

Cyanide sufficient to excite ventilation when injected intravenously was found to produce no demonstrable effects when injected at the same rate into a patent common carotid artery. If prior to injection the opposite carotid or the vertebral arteries were occluded, injection into the patent carotid artery excited ventilation. This excitation was in turn prevented by occlusion of the corresponding internal carotid.

Injection of cyanide into the patent vertebral arteries elicited marked effects,—first stimulation followed by depression which was difficult to avoid.

It is concluded that any procedure which shunts or delivers cyanide to the lower nerve centers tends to excite ventilation while any procedure which confines the delivery of cyanide to the head region excluding the lower nerve centers is unaccompanied by excitation.

It, therefore, appears that chemical stimulating effects of cyanide in the head region are largely if not entirely confined to nerve centers rather than to a peripheral nervous mechanism.

The results are in agreement with other experiments on peripheral chemical stimulation of the vagus nerve endings by disturbances of oxidations.

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THE DIURETIC-ANTIDIURETIC EFFECT OF THE PRESSOR PRINCIPLE OF THE POSTERIOR LOBE OF THE PITUITARY GLAND[1]

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The posterior lobe of the pituitary gland contains two important active principles, one which causes uterine contractions and one which raises blood pressure. These active principles have been separated and obtained in nearly pure form by Dr. O. Kamm, Dr. T. B. Aldrich, Dr. I. W. Grote, Mr. L. W. Rowe and ourselves. This work was reported by Kamm at a meeting of the American Chemical Society, September 7, 1927, and has recently been published in detail so that others can repeat the work and obtain the same results (1928).

The principle which contracts uterine muscle has been named alpha-hypophamine and the principle which raises blood pressure, beta-hypophamine. As the names indicate, both active principles are believed to be amines because of their chemical behavior.

After it had been established that there were two active principles in the posterior lobe of the pituitary gland it interested us to know if the well known diuretic-antidiuretic action of pituitary extracts was due to one of these principles or due to a third active principle.

In investigating this point rabbits were used and the method of experiment was similar to that originally employed by Magnus and Schafer (1901), Schafer and Herring (1906, 1908) and Houghton and Merrill (1908) on dogs; and more recently on rabbits by Abel, Rouiller and Geiling (1923), Mackersie (1924), Smith and McClosky (1924) and others.

Normal rabbits weighing about 2 kgm. were fed on oats and green food with plenty of water always available. The rabbits were anesthetized with urethane, amytal or chloral hydrate by subcutaneous injection in dilute aqueous solution or with chloretone by intraperitoneal injection in alcoholic solution. A glass cannula was tied into the urinary bladder and then the rabbit was placed on a board with the cannula extending vertically downward through a hole in the board. This position of the rabbit favored the free flowing of urine. Pocketing of urine was prevented by

[1] Paper read before the Federation of American Societies for Experimental Biology, April 14, 1928.

having a funnel-shaped end on the cannula. Capillary action was reduced to a negligible quantity by using a large cannula, about 1 cm. in diameter, with an obliquely pointed lower end.

The rate of urinary flow was determined by observing the number of drops of urine falling in 5 minute intervals. After a control period of half an hour or more, various dosages of extract of desiccated posterior lobes (Pituitrin), solution containing the uterine muscle contracting principle (Oxytocin), and solution containing the blood pressure raising principle (Vasopressin), were given by injection into the marginal ear vein of the rabbit.

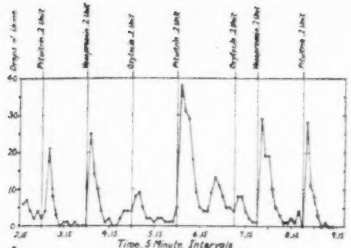
The solutions employed had all been standardized by both the oxytocic method and the blood pressure method so their activities were known with considerable accuracy.

The Pharmacopoeia of the United States recognizes only the oxytocic test as official. A solution 100 per cent standard by the oxytocic test contains 10 International Units (League of Nations 1925) of the oxytocic active principle per cubic centimeter. It has been noted by Kamm, Aldrich, Grote, Rowe and Bugbee (1928, p. 579) that desiccated posterior lobes of the pituitary gland yield extracts which are remarkably constant in their relative potency by oxytocic and pressor tests. This fact has led them to adopt as a pressor standard the solution which the Pharmacopoeia has used for the oxytocic standard, that is 1 cc. containing the activity of 5 mgm. of Standard Powdered Pituitary. This solution contains 10 units of oxytocic activity and also 10 units of pressor activity. Of the solutions used in these experiments; Pituitrin tested 100 per cent oxytocic and 100 per cent pressor; Oxytocin tested 100 per cent oxytocic and 4 per cent pressor; and Vasopressin tested 100 per cent pressor and 8 per cent oxytocic. The Oxytocin and Vasopressin were parts of large lots which had been sent out to several other experimenters and did not represent as complete separation of active principles as had been made in smaller lots where the separation had been greater than 99 per cent complete.

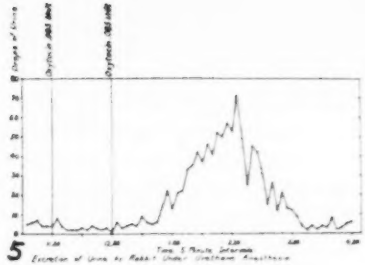
Excretion of urine. Figure 1 shows the excretion of urine by rabbit 2 weighing 2.1 kgm. under urethane anesthesia 2 grams per kilogram. In the half-hour control period the urine flowed at the rate of from 2 to 7 drops every 5 minutes

When 0.2 unit of Pituitrin was injected there was first a short latent period or possibly a short period of suppression of urine followed by an increased excretion of urine amounting to 21 drops in 5 minutes. Then followed the antidiuretic effect so that in 40 minutes only 3 drops of urine were excreted.

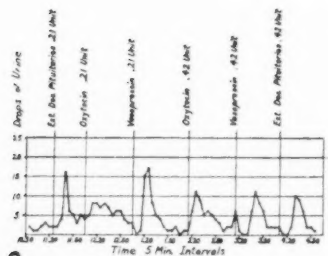
When 0.2 unit of Vasopressin was injected there was a flow of urine amounting to 25 drops in 5 minutes. This was followed by the anti-



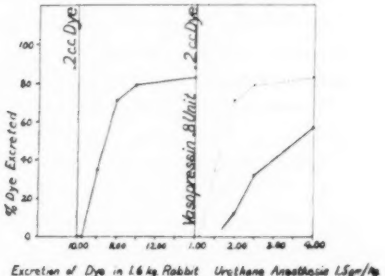
1 Excretion of Urine by Rabbit Under Urethane Anesthesia



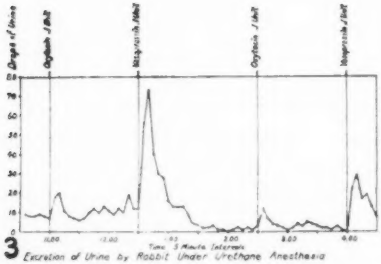
5 Excretion of Urine by Rabbit Under Urethane Anesthesia



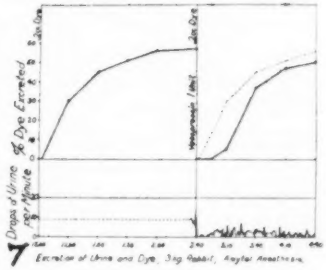
2 Excretion of Urine by Rabbit Under Urethane Anesthesia



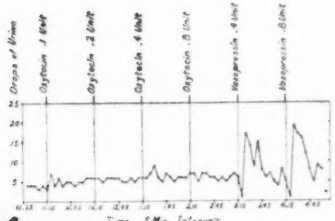
6 Excretion of Dye in 1.6 kg. Rabbit Urethane Anesthesia 1.5gr/kg
.8 Unit of Vasopressin Injected Intravenously



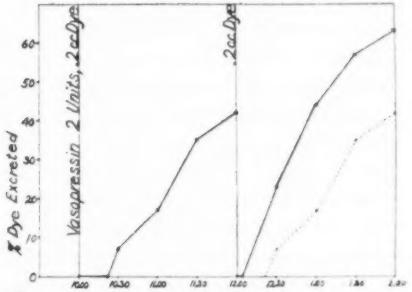
3 Excretion of Urine by Rabbit Under Urethane Anesthesia



7 Excretion of Urine and Dye, 3 kg. Rabbit, Amytal Anesthesia



4 Excretion of Urine by Rabbit Under Urethane Anesthesia



8 Excretion of Dye in 2 kg. Rabbit, Amytal Anesthesia 80 mg. per Kg.
2 Units of Vasopressin Injected Intravenously

Figs. 1–8

diuretic effect. No urine was excreted in two 5-minute periods. The antidiuretic effect was subsiding when 0.2 unit of Oxytocin was injected. This was followed by slight increase of flow of urine which may have been due to a small amount of Vasopressin in the solution, about 0.01 unit.

The injection of 0.2 unit of Pituitrin caused a gush of urine which reached the rate of 38 drops in 5 minutes and lasted for 20 minutes. The antidiuretic effect was not apparent after this injection.

The injection of 0.2 unit of Oxytocin produced very little effect.

The injection of 0.2 unit of Vasopressin on the other hand caused diuresis amounting to 29 drops in 5 minutes. This diuresis subsided so that in the course of 35 minutes the antidiuresis began to be apparent.

An injection of 0.2 unit of Pituitrin caused a gush of urine, 28 drops being excreted in 5 minutes. The antidiuretic effect appeared in 25 minutes.

Figure 2 shows the excretion of urine by rabbit 4 weighing 2.2 kgm., under urethane anesthesia 2 grams per kilogram. Attention is called to the antidiuretic effect which is apparently the first effect of injections of Vasopressin and Extract of Desiccated Pituitaries. This early effect has been ascribed to spasm of the ureters by Mackersie (1924). It lasts 10 or 15 minutes and is followed by a diuretic effect lasting 10 to 20 minutes.

Figure 3 records an experiment with rabbit 15 weighing 20 kgm., under urethane anesthesia 2 grams per kilogram. It shows the very slight effect from the injection of 0.1 unit of Oxytocin. The injection of 0.1 unit of Vasopressin, however, is followed by a considerable flow of urine, 73 drops in 5 minutes. This flow subsided in the course of 1 hour and very little urine was excreted for the next hour.

Figure 4 records an experiment with rabbit 5, weighing 2.0 kgm. under urethane anesthesia 2 grams per kilogram. It shows quite definitely the *lack* of diuretic-antidiuretic action of even 0.8 unit of Oxytocin.

The injection of 0.4 and 0.8 unit of Vasopressin was followed by an immediate antidiuretic effect followed by a diuretic effect. Probably a subsequent antidiuretic effect would have been observed if the experiment had been continued longer.

Figure 5 shows the tremendous flow of urine noted in one rabbit (no. 7, weight 1.7 kgm., urethane 2 grams per kilogram). Apparently it had no connection with the small doses of Oxytocin which had been injected earlier in the day. No explanation is offered for this great flow of urine for no similar thing happened in any of the other 87 rabbits used in this series. It is not probable that pocketing of urine had taken place in the bladder for if such were the case the pocket should be emptied in a few seconds whereas this flow lasted for 2 hours.

Excretion of dye. After it has been found by experiment that the pressor principle has the diuretic-antidiuretic activity it interested us to know if this action was upon the water secreting mechanism alone or upon the salt

secreting mechanism of the kidney. To test out this point intravenous injections of the dye phenolsulphonephthalein were given to rabbits. The method was similar to the clinical renal function test. Two-tenths cubic centimeter of phenolsulphonephthalein was injected into the marginal ear vein of a rabbit under anesthesia. For this purpose amytal, urethane and chloral hydrate were used.

The urine was collected and tested for the amount of dye excreted in ½ hour, 1 hour, 1½ hour and 2 hour intervals. Then a second injection of the dye was given and a dose of either Oxytocin or Vasopressin was given. The amounts of dye excreted after these drugs were compared with amounts excreted during the previous control periods. In a few experiments the order was reversed and the drug was given in the first period and the second period served as control.

Figure 6 records the experiment with rabbit 53 weighing 1.6 kgm. under urethane anesthesia 1.5 grams per kilogram. In the control period the dye first appeared in 7 minutes. At ½ hour, 1 hour, 1½ hours and 3 hours the excretion of dye was 35 per cent, 71 per cent, 79 per cent and 83 per cent. After 0.8 unit of Vasopressin had been injected the dye did not appear for more than ½ hour. The excretion at 1 hour, 1½ hours and 3 hours was 12 per cent, 32 per cent and 57 per cent. For comparison the control period is shown in dotted lines, superimposed.

Figure 7 shows the experiment with a 3 kilogram rabbit (no. 64 under amytal anesthesia 75 mgm. per kilogram) in which the urine flowed the most freely of any rabbit in the whole series. In the control period it averaged 9 drops per minute (counted by an electrical drop counter and recorded on the kymograph).

When 1 unit of Vasopressin was injected the flow of urine stopped and then took place at a much reduced rate. It is to be noted that no diuresis occurred but only the antidiuretic effect.

The excretion of dye was moderately great in the control period. The first drop of dye appeared in 3 minutes. At ½ hour, 1 hour, 1½ hour, 2 hour and 3 hour intervals the excretion was 30 per cent, 45 per cent, 51 per cent, 56 per cent and 57 per cent.

After 1 unit of Vasopressin had been given the first drop of dye appeared in 14 minutes. At ½ hour, 1 hour, 1½ hours and 2 hours the excretion of dye was 5 per cent, 37 per cent, 47 per cent and 50 per cent. The control period is shown in dotted lines, superimposed.

Figure 8 shows the effect of injection of 2 units of Vasopressin (rabbit 71, 1.9 kgm., amytal 80 mgm. per kgm.). The dye first appeared in 22 minutes. At ½ hour, the excretion of the dye was 7 per cent, in 1 hour 17 per cent, in 1½ hours 35 per cent and in 2 hours 42 per cent.

In the subsequent control period the dye first appeared in 4 minutes. The excretion of dye in ½ hour was 23 per cent, in 1 hour 44 per cent, in

1½ hours 57 per cent, and in 2 hours 63 per cent. The dotted line shows the excretion in the Vasopressin period superimposed for comparison.

These three experiments seem to indicate quite definitely that Vasopressin decreases the excretion of dye. The experiments with Oxytocin on the other hand seem to indicate very little effect on the excretion of dye.

Table 1 shows the summary of the experiments on the excretion of dye. Seven experiments with Oxytocin showed a slight decrease of excretion while 6 experiments showed a slight increase. In no experiment was the change in excretion definite enough to warrant the conclusion that Oxytocin had produced a noteworthy effect.

These experiments have not given us any information regarding the kidney mechanism affected by the pressor principle of the pituitary. It has not been evident whether the rate of excretion of dye has been affected independently or whether its rate has always been secondary to the rate of excretion of water. The dye cannot appear in the urine except

TABLE 1

Diuresis experiments. Excretion of phenolsulphonephthalein. Rabbits under anesthesia

	DECREASE	NOT MUCH EFFECT	INCREASE
Oxytocin	7	6	6
Vasopressin	12	4	2

as it is carried along by the water. On the other hand water may be excreted without dye being excreted. In three experiments this actually happened, urine was excreted for several hours without any of the dye appearing.

In these diuresis experiments 88 rabbits have been used. Only a small proportion of the experiments are reported but they are representative. In many experiments there was such a poor flow of urine in the control period that the animals had to be discarded.

The depth of anesthesia seemed to vary greatly with different animals under equal dosage per kilogram of body weight. It is probable that the depth of anesthesia was the chief factor in causing such wide variation in the amount of urine excreted during preliminary observation periods.

SUMMARY AND CONCLUSIONS

The pressor principle of the posterior lobe of the pituitary gland has both a diuretic and an antidiuretic effect on green fed rabbits under anesthesia.

The oxytocic principle has very little if any diuretic-antidiuretic action on anesthetized rabbits.

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A NOTE ON ACTION CURRENTS AND "EQUILIBRATION" IN THE CAT'S PERONEAL NERVE

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Although the teaching of "non-fatiguability of nerve fibres" has remained for forty years a major landmark in physiological thought, investigators studying action currents have for a long time found considerable evidence to the contrary. Perhaps the most complete of the older studies is that of Tigerstedt (1912) in which he showed a fall of the maximum and prolongation of the descending limb of the action currents of a frog's sciatic as a result of continued activity.

More recently, it has been demonstrated by Field and Brücke (1926) that with minutes of continued stimulation the refractory period of the frog's sciatic is greatly prolonged, and Gerard (1927a and b) has found that oxygen consumption, heat production and action potential of this nerve become much less on continued than on intermittent stimulation. These results seem to prove, for this variety of nerve, that activity lessens for a matter of minutes the capacity for further activity. In the case of certain non-medullated nerves—the pike's olfactory (Garten, 1903) and the spider-crab's leg (Levin, 1927) nerve—it has been shown that continued activity leads to progressively feebler responses and ultimate inability to conduct, quite analogously to the gradual failure of a repeated voluntary contraction. This progressive fall and ultimate loss of ability to act, accompanying continued activity, is probably what is most generally understood by the word "fatigue."

In the frog's sciatic the fall in activity begins at once at a maximum rate and continues at a diminishing rate only for a matter of minutes, after which the level reached is maintained for hours, provided the stimulation is not modified. An increase in the number of stimuli in unit time causes this level to fall, a decrease in stimulation causes it to rise to some new level which is again maintained. This phenomenon, being different from that understood by fatigue, has been called equilibration (Gerard, 1927b), and a theoretical interpretation of it has been advanced (Gerard, 1927c). Most experiments designed to test for nerve fatigue depended on the presence or absence of contraction of an innervated muscle to demonstrate the functional condition of the nerve, and since the equilibration level is

always above zero (because of the existence of an absolutely refractory phase), some impulses necessarily would reach the muscle, when a block is removed, and cause a contraction. Relatively few impulses per second are required for a complete tetanus, and any subnormal impulses in the traversed stretch of nerve would presumably return to normal value on entering the fresh nerve peripheral to the portion previously blocked, so that the phenomenon of equilibration would be entirely masked in such experiments.

The observations to be described were obtained partly incidentally to the experiments reported in the following paper. They are only fragments, but are sufficient, we believe, to show that, as evidenced by its action currents, the cat's peroneal nerve exhibits phenomena similar to those of the frog's sciatic.

METHODS. These are more fully described in the succeeding and in earlier communications. The wiring plan was essentially as shown by Forbes and Gregg (1915); the recording camera was described by Forbes and Thacher (1920). The excised peroneal nerve of a decerebrate cat, dissected free from the popliteal nerve from hip to knee, was led over silver stimulating electrodes to rest on two boot electrodes in a moist chamber. These leads were over 20 mm. apart and the nerve was usually crushed between them for monophasic recording. The stimulating electrodes were about 3 mm. apart and 20 to 25 mm. proximal to the nearest lead. The arrangement was the same as that used in recording reflex motor nerve impulses, reported in the following paper, except that the nerve was cut at the hip to eliminate confusion due to reflex impulses. The temperature of the nerve on the various electrodes was about 28°C. in all experiments.

Ascending induction shocks (sometimes make and break, sometimes only break) from coreless Harvard coils, arranged in accordance with the principles set forth by Bishop, Erlanger and Gasser (1926) to minimize shock escape, were used for stimulation. Single shocks or short series were given by hand, longer series and high frequencies were obtained with the rotating commutator (Forbes, 1921; Querido, 1924).

The action currents were led from the boots via NaCl, $ZnSO_4$, Zn, to a Hindle galvanometer with 1.5 mm. air gap. The string was gilded quartz, resistance = 19,500 ohms.

RESULTS. *a. The single action current.* The records reproduced in figure 1 serve to demonstrate how variable the action current following a single maximal induction shock may be. The descending limb is especially inconstant, exhibiting, angles, humps, peaks, negative remainders and positive after-variations, often lasting several tenths of a second, as well as the classical rapid and smooth return. Essentially similar observations have been previously reported from this laboratory (Davis and

Brunswick, 1926), but these more or less aberrant action currents were in many cases so striking and constant that before the nerves were cut centrally we were misled into considering the peaks and humps as due to secondary action currents produced by reflexes. The crucial test in all cases was, of course, the severing of the central connections, which abolished any true reflex effect, and only such effects as were at once abolished by this procedure were considered in the work on reflexes reported in the

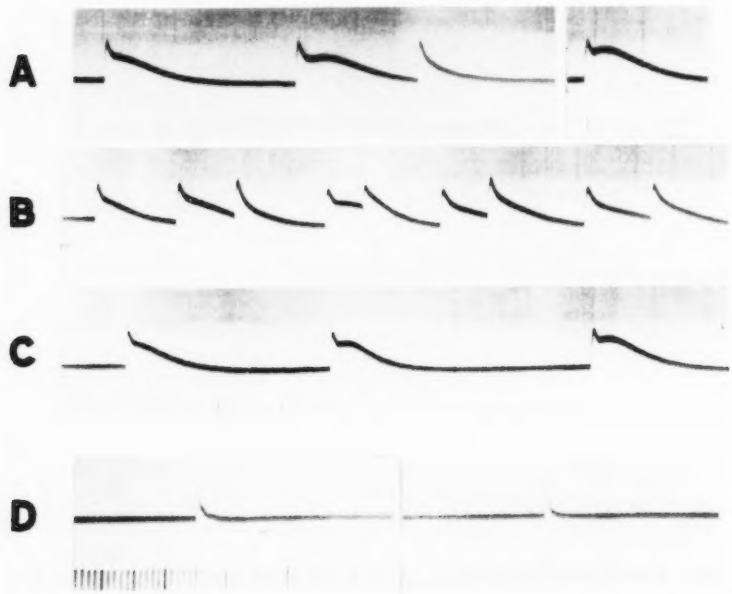

Fig. 1. Galvanometer records of action currents showing variability, and especially the changes correlated with frequent stimulation. Magnification in all, 490. Time below in 0.01 sec. intervals. A, B and C, November 9, 1927. Nerve completely isolated from animal. String tension, 640 M. per amp. D, November 1, 1927. 1, before severing nerve at the hip; 2, after severing. The humps following the main peak in no. 1 show the reflex response to the stimulus. String tension, 200 M. per amp.

following paper. An example of the reflex effect which disappears on cutting the nerve centrally is shown in figure 1, D.

The factors responsible for these various forms of action current have not been analysed. In general any one preparation tended to exhibit one type from the start with, possibly, a gradual change during the course of the experiment (4 to 6 hrs.) to another type. The conditions of preparation, the electrical circuits, the recording apparatus, etc., were essentially

constant in all experiments and we feel convinced that the action currents recorded represent, though with distortion, genuine effects dependent on the activity of the nerves, and therefore physiologically significant. More definite evidence for this view will appear below.

b. Short series. Several definite changes in the shape of the action current for successive impulses occurred when several were elicited in a series, even when they occurred at such slow frequencies as to allow several tenths of a second between individual stimuli (see fig. 1, also fig. 4, F in the following paper).

1. The first one or several responses were less than later ones, as measured by the maximum displacement of the string. This occurred with definitely supramaximal stimuli and was, therefore, not due to a lowering of threshold of some fibres. Similar effects have been recently reported by Davis and Brunswick (1926) and by Gerard (1927a) and very early by Waller (1897). When stimuli were thought to be just maximal this rise was sometimes more marked, suggesting a "recruitment" of fibres with repeated stimulation.

2. With rapid stimulation there appeared, after an initial increase, a slight fall in the maximum displacement of the string.

3. Angles, humps and other divergences of the descending limb from the usual smooth curve tended to disappear with repeated activity, and to return with rest. This smoothing out was greatest with more rapid stimulation, but was complete after 4 or 5 responses even at an interval of 100 to 200σ and between shocks.

c. Continued tetanization at high frequencies. Nerves were tetanized continuously at 150 to 500 shocks a second for 1 to 6 minutes and records of the action currents taken at 30 second intervals during stimulation and also during several minutes after tetanization was stopped (by resuming stimulation for a few seconds while the records were being made). During the time records were taken the strength of stimulus was altered once or twice by rapidly changing the resistance in the primary coil. Samples of the resulting records are shown in figure 2. Responses to initially slightly supramaximal stimuli rapidly became irregular and later were reduced to one every second stimulus (the make shocks becoming ineffective). Further tetanization again brought irregularity or regular alternation of large and small impulses, and finally responses were obtained only to every fourth stimulus. When the nerve was responding irregularly to stimuli or regularly to some sub-multiple, a considerable increase in strength of the stimuli would bring back a regular response at the true frequency. Often when the individual responses were irregular, an appearance of regular beats was noted, as if two separate frequencies were present together.

In a similar way during several minutes of rest following a long tetaniza-

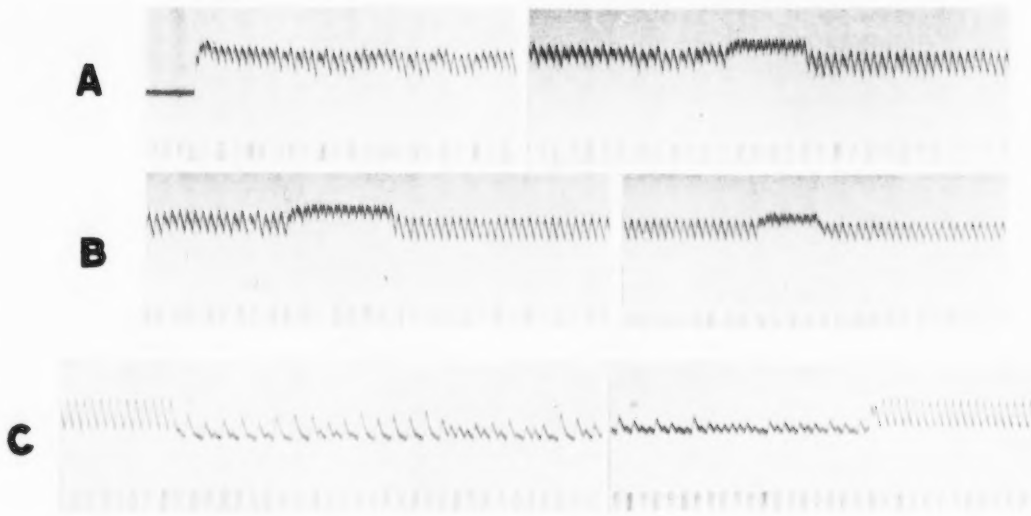

Fig. 2. Records showing effects of prolonged tetanizing, and especially of changing the strength of stimuli at various stages of "equilibration." Time shown below in 0.01 sec. intervals. A and B, November 3, 1927. String tension, 240 M. per amp. A1, beginning of stimulation; moderate strength of stimulus (250 ohms in primary circuit) make shocks almost cease to excite before end of record. A2, 1.6 sec. after beginning continuous tetanization with 500 stimuli per sec. (counting make shocks) with 50 ohms in primary. Strength of stimulus is altered for a fraction of a second to observe effect; record begins with 150 ohms, shows change to 50 ohms and second change to 250 ohms, rendering makes ineffective. B1, same procedure after 30 seconds of continuous tetanizing with 50 ohms in primary. B2, same procedure after 1½ minute of tetanizing. C, October 28, 1927. String tension, 320 M. per amp. 1, 1.5 sec. after beginning of prolonged tetanization with 140 ohms in primary, occasionally changed for brief time to 40 ohms. Record begins with 40 ohms, changed during record to 140. Complete rhythm reappears after partial restoration from lower level of equilibration. 2, after 5 minutes' continuous stimulation and rest of 5 minutes; change from 140 ohms to 40 ohms.

tion, the nerve responded better at successive tests until the original condition was regained. A particularly good demonstration of this recovery was obtained in the following way. The tetanization was performed with strongly supramaximal stimuli so that all continued to be effective, and then, while the tetanization continued, the stimulus strength was reduced to a value previously found to be slightly supramaximal for the rested nerve. At first the alternate stimuli remained ineffective, then responses began to follow every stimulus.

These facts indicate a rise in threshold with stimulation, which can perhaps most simply be understood in terms of the relative refractory period. The repeated activity, by depleting some factor necessary for the immediate recovery process, leads to progressively slower recovery from each conduction until a new equilibrium value is reached (see Gerard, 1927c). At any given frequency, therefore, each stimulus finds the nerve progressively earlier in its relative refractory phase and with a correspondingly raised threshold. An originally maximal stimulus becomes subminimal to any fibre which it finds in this state and fails to stimulate, the fibre continues its recovery and may then be excited by the following stimulus. Since the various individual fibres differ from one another in the exact relations of threshold and recovery, they will soon be out of phase and the summated response of the whole nerve will be very irregular, though each stimulus continues to be effective. Further tetanization and rise of threshold causes make shocks to become subminimal to all fibres and regular responses at half the frequency result. As the immediate recovery becomes more delayed the break shocks, even at twice the original interval, become subminimal to some fibres and irregularity again results.

It is not so easy to understand why further stimulation again leads to alternation or halving the frequency of response, for even if each fibre responds only to every second stimulus, half might be expected to respond to the odd shocks and half to the even ones, rather than all or most of the fibres to the odd or the even ones. The former condition would lead to a regular response of half normal intensity but at the full frequency, and this may have in fact occurred in some cases. With lower frequencies of tetanization the nerve followed the stimuli better than with high frequencies, which fact is in consonance with the interpretation given.

Discussion. These several observations agree in demonstrating that activity of a nerve definitely modifies its capacity for action. The changes in the descending limb of the action current, as well as the increased threshold accompanying repeated activity, may be taken as evidence of change in the events occurring during the refractory period, that is, the immediate recovery from each response. These changes are progressive to only a certain degree as activity continues and are reversed during inactivity. They indicate that even when a nerve has recovered its ability to conduct,

following conduction of one impulse, the return to the resting state is not yet complete; that is, a delayed recovery lasting some time must follow the immediate recovery, and failing this the latter is interfered with. These results, therefore, are in accord with the previous experiments on frog nerve and the interpretation given them. It must be noted, however, that the effects of prolonged tetanization of the cat's peroneal are less pronounced than in the case of the frog's sciatic. We have not attempted a comprehensive study of the cat's nerve from this aspect, and it is possible that under other conditions of stimulation than those used the effect would have been brought out more strikingly. In any event the differences noted are of degree and not of kind.

The bases of the various shapes of action currents observed have not been elucidated. Since they are essentially constant for one preparation and are definitely modified by activity or rest of the nerve, we believe them to be genuine biological effects. Changes in resistance of the various elements and in the local potentials developed could account for the effects, and these in turn probably rest on definite chemical change associated with nerve activity. It is impossible for the present to associate them with changes in the concentration of oxygen, carbon dioxide or any other specific substance, though one of these gases may be especially concerned.

SUMMARY

1. Aberrant action currents are often obtained from the isolated cat's peroneal nerve. The descending limb, especially, may exhibit angles, humps, peaks and negative or positive remainders. These are often so marked that in a nerve connected with the spinal cord, they may be mistaken for reflexes. They tend to disappear when the nerve is repeatedly active, even at intervals of several tenths of a second, and return with inactivity.

2. Continued tetanization of a nerve for several minutes with 150 to 500 stimuli a second causes a gradual failure to respond at these frequencies to stimuli which were at first slightly supramaximal. Strongly supramaximal stimuli may still all be effective. Inactivity for several minutes restores the initial state.

3. The cat's peroneal nerve, therefore, is not unfatiguable; it undergoes definite changes as a result of activity which are not entirely reversed during the refractory periods but only gradually disappear during several minutes of rest. This lag in recovery leads to "equilibration" as a result of activity; in this respect the nerve resembles that of the frog.

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"FATIGUE" OF THE FLEXION REFLEX

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Forbes and Gregg (1915), in their first studies of the flexion reflex in the decerebrate cat, observed that in the peroneal nerve the action current of the reflex response to single maximal stimuli remained constant only when the individual shocks were delivered at adequate intervals. When stimulation was repeated at intervals of a few tenths of a second, the electric response of the motor nerve fell off considerably during the first few responses and then remained constant at some lower level. This early but partial fall did not resemble a typical fatigue effect, and Forbes (1922) and Forbes, Cobb and Cattell (1923) later advanced a different interpretation of the phenomenon.

It was pointed out that after-discharge of considerable magnitude persists after a single flexion reflex has been elicited, that this presumably means a continued activity by some of the motor neurones for a variable but considerable time after the onset of the reflex, and that consequently afferent impulses produced by a second shock within this time would arrive at motor neurones some of which were preoccupied and, therefore unable to respond to them. This "line-busy" effect, as it has been called, would account for a falling off of successive responses for a short time. In accord with this view, it was further found that low transection of the spinal cord diminished this falling-off of successive responses (and increased the absolute magnitude of all responses), and that the mechanically recorded after-discharge of the reflex was also decreased. The shorter after-discharge, decreased "line-busy" effect, and increased amplitude were regarded as resulting from release of the spinal centers from descending impulses, largely generated by afferent stimuli travelling to the higher centers and down again via "delay paths."

McCouch (1924) has pointed out certain difficulties in this explanation. Sherrington and Sowton (1915) observed an immediate increase in the flexion reflex to a single shock on spinal transection of a previously decerebrated cat, but even earlier Sherrington (1910) had observed the same effect with tetanizing stimuli. McCouch also presents evidence for regarding the flexion reflex as being largely under the influence of descending cortical tracts, and for regarding spinal shock as the result of interrupting

them. Since these tracts are already severed by decerebration, subsequent spinal section could hardly be expected to manifest the typical effects which are observed when the cord is transected in the intact animal. The fact remains, however, that in the decerebrate cat section of the spinal cord does lead to an increased flexion reflex, measured by the nerve action currents or the muscle response, and that the diminution in successive responses is less marked.

Gerard (1927a and b) has recently observed in peripheral nerves a phenomenon somewhat similar to the successive diminution of flexion reflex responses. The magnitudes of the action current, heat produced and oxygen consumed by a nerve during activity, fall rapidly with successive short tetanizations. The fall is not to extinction, but to some equilibrium level which is lower with tetanizations repeated at shorter intervals or continuous at higher frequencies. This early but partial fall, which has been called "equilibration" is, qualitatively at least, similar to the fall in reflex action currents. A possible explanation of this has been advanced (Gerard, 1927c) and similar effects in the cat's peroneal have been described in the preceding paper (Gerard and Forbes, 1928).

It seemed worth while to us to attempt to determine experimentally how much of the fall in reflex response with repetition was due to the "line-busy" effect and how much to "fatigue" or "equilibration."

METHODS. For this analysis a method very similar to that used by Forbes, Whitaker and Fulton (1927) was quite suitable. Stimulating electrodes were placed on the central end of the cut popliteal nerve of one leg of a decerebrate cat, to elicit a reflex flexion response of the tibialis anticus muscle. Similar electrodes were also placed on the intact peroneal nerve of the same leg, which carries the reflex motor impulses, so that maximal stimuli could be delivered at will. With the motor centers at rest, a maximal stimulus should find all the fibres in the peroneal nerve inactive and should, therefore, cause a constant, maximal response. Any activity of the motor centers, with attendant conduction of impulses down their axons in the peroneal nerve, would render these fibres inaccessible to further stimulation by a shock along their course, and the response to such a shock would, therefore, be reduced. The size of response was measured by the action current led off monophasically (occasionally diphasically) from the peroneal nerve near the knee.

This method has one serious limitation. The direct stimulus excites all motor and sensory fibres in the nerve trunk simultaneously, and the impulses are essentially in phase a few centimeters away, where the action current is led off. The galvanometer deflection is, therefore, maximum. The reflex response does not, of course, activate the sensory fibres in the peroneal, and only a fraction, often a small one, of the motor fibres. Further, the discharges, and still more the after-discharges, of

the motor cells are largely asynchronous. The observable maximum action current of the reflex is, therefore, only a small fraction of that of the direct stimulus. In our experience this fraction was seldom greater than 10 to 15 per cent. A direct stimulus thrown in at the height of a reflex response would, therefore, give an action current reduced only by this per cent compared to that obtained from a resting nerve. Our purpose was to detect the persistence of activity in only a small fraction of the fibres involved in the reflex, so the reduction to be anticipated in the direct response was even smaller. The problem resolved itself, therefore, to the detection of changes of a few tenths of a millimeter in the height of a string deflection of five to ten millimeters, and accurate quantitative re-

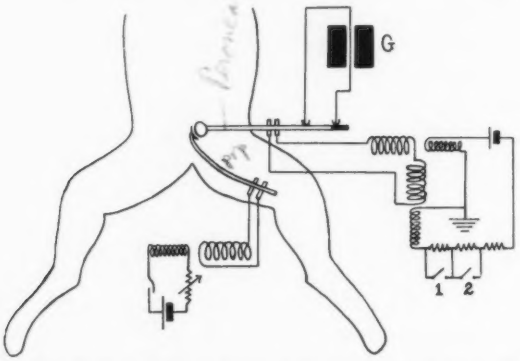

Fig. 1. Diagram of arrangement of preparation and apparatus. The stimulating circuit with the single inductorium is shown connected with the popliteal (afferent) nerve. The stimulating circuit with the balanced coils is connected with the peroneal (motor) nerve, from which action currents are led off to the galvanometer, *G*. The arrangement is shown whereby with the latter stimulating circuit two equal stimuli can be applied by opening the keys (1 and 2) of the pendulum, successively breaking the short-circuits of two resistances.

In some experiments the two keys of the pendulum were arranged to stimulate first the popliteal, then the peroneal nerve; i.e., key 1 was introduced into the first primary circuit, key 2 into the second in series with the resistances.

sults could not be expected. Within reasonable limits, however, the results were quite consistent and permit definite conclusions to be drawn. The direct response could be reduced by cutting posterior roots and allowing time for degeneration before the acute experiment, and by leading from the peroneal branch to the tibialis anticus rather than the whole nerve. Both methods were tried but were not found very practical.

The usual experiment involved delivering a single maximal break shock to the popliteal, followed at varying intervals by a similar shock to the peroneal. For spacing the stimuli either a series delivered by hand, a

swinging key (Forbes, Querido, Whitaker and Hurxthal, 1928) or the Lucas pendulum was used, the two shocks being obtained from separate coils or from the same coil by throwing two resistances in succession into the primary circuit. A modified method was also used. Since the direct shock applied to the peroneal excites both sensory and motor fibres, a reflex is elicited by such a stimulus as well as by one to the popliteal. The action current led off peripherally shows, therefore, the sharp spike of the direct response and, on the falling limb of this, a small but definite reflex peak. By using a pair or series of direct stimuli to the peroneal it is possible to make the same experiment as by first stimulating the popliteal and later the peroneal. As will be seen, these experiments afford an especially concise means of distinguishing between "line-busy" and "fatigue" effects.

Besides the direct determination of the duration of the "line-busy" effect we have determined, by mechanical records from the foot recording contraction of the tibialis anticus muscle, the duration of after-discharge of the flexion reflex. This was done after all the dissection was complete and just before the peroneal was cut and arranged on the electrodes.

We have also more carefully observed the decline in the reflex response on repetition and determined the interval between successive stimuli which will produce it. For this purpose a series of stimuli to the popliteal nerve was delivered at various frequencies by hand and with the rotary interrupter (Forbes, 1921; Querido, 1924).

EXPERIMENTAL DETAILS. *a. Preparation of the cat.* The cat was decerebrated under deep ether anesthesia by the guillotine method. Exposure of the spinal cord in the lower thoracic region, when performed, was done just prior to the decerebration. Following decerebration, a skin incision in the right thigh was made and the muscles about the hip joint severed, but the nerves were not exposed until two or three hours later when the acute effects of the operation were past and the experiment could be continued. The peroneal and popliteal nerves were then separated to the hip, the nerve to the hamstrings cut, the popliteal cut and the central end led over shielded stimulating electrodes, and the femur doubly clamped. In several experiments the sciatic nerve of the left leg was cut and the central end arranged for stimulation. In one successful experiment the lower right leg was de-afferented by section of the last two lumbar and first sacral posterior roots. Twelve days later, when used for the usual experiment, the lower leg was paralyzed and anesthetic, and after the preparation was made no reflex effects were obtained on cutting or electrically stimulating the sciatic on the operated side. For reflex stimulation in this animal the anterior crural nerve was prepared instead of the popliteal, both on the de-afferented and on the normal side, which was subsequently studied as a control on the first.

b. Mechanical records. When the preparation was complete but for the cutting peripherally of the peroneal nerve, the ankle was lightly clamped and the foot attached to a lever writing on a smoked drum. Reflex contraction of the tibialis anticus was thus recorded.

c. Leads for action current. Following these records, the peroneal nerve was

cut distally and led into a moist chamber containing two stimulating electrodes (platinum or silver) and two leading-off, boot electrodes (Zn-$ZnSO_4$-Ringer). The stimulating electrodes were about 20 mm. proximal to the nearest lead and themselves 2 to 3 mm. apart. The distal lead was 20 mm. beyond the proximal one, and the nerve was usually crushed between them, to render the action current monophasic.

d. Electrical records. The Hindle galvanometer with 1.5 mm. air gap was used and the circuits were essentially as previously described (Forbes and Gregg, 1915). In several experiments the electron-tube amplifier (Forbes and Thacher, 1920) was included. A gilded quartz string of 19,500 ohms resistance was used throughout at tensions of 240 to 640 meters per amp. (Forbes and Ray, 1923).

e. Stimulation. Two coreless Harvard induction coils were used for stimulating the peroneal nerve, arranged, as described in our previous paper, to minimize escape of current into the galvanometer circuit. The secondaries were directly over the primaries and the strength of stimulus adjusted by a resistance in the primary circuit. A single coreless coil was used for the reflex stimulation. Single shocks were given by hand, as were also short series at relatively low frequencies. Long or rapid series were delivered by the rotary interrupter, which could be included in the primary circuit by a switch. For delivering two shocks at given intervals to the same or different nerves, several methods were used. For a certain range a swinging double key, the two keys opened in succession and each breaking one primary circuit, was quite satisfactory. More widely useful was the Lucas pendulum arranged also to break two primary circuits in succession. When two shocks were to be delivered to the peroneal, it was not considered satisfactory to use separate coils, and the following arrangement was used with the standard stimulating circuit. In the primary circuit were placed three resistances, two short-circuited through the two keys on the Lucas pendulum. The swing of the pendulum broke these short circuits at desired intervals thus throwing into the primary circuit, in addition to the first resistance already present, the two other resistances at the given interval. Each sudden fall of current in the primary, as the resistances were added, gave an induced shock. By choosing the resistances of appropriate ratio (2:1:3 for the permanent resistance, the first and second ones added, respectively) the two shocks were made equally strong, and the intensity of both was varied by changing the voltage in the primary circuit or the actual values of the three resistances. The arrangement is shown in figure 1.

RESULTS. *a. Mechanical evidence of after-discharge.* In six experiments the after-discharge of the flexion reflex, measured by the maintained rise of the lever attached to the cat's foot, was observed. In one of these the reflexes evoked by a single afferent stimulus or a short series showed no measurable after-discharge; the method used would not reveal a very short one. In all others a rather prolonged after-discharge was observed, lasting from $\frac{1}{2}$ to $1\frac{1}{2}$ second after a single shock and from 2 to over 4 seconds after a short series. The amount of after-discharge, after the first fraction of a second, was in all but one only a small per cent of the total reflex response, measured by the displacement of the lever (see fig. 2). Sherrington (1921) gives similar figures for the after-contraction of the cat's tibialis anticus following a single shock to the ipsilateral internal saphenus nerve.

This seems to afford evidence that some of the motor neurones continue

to discharge for a considerable time after a flexion reflex has been elicited by a single shock, and these would be refractory to a fresh stimulus impinging on them from afferent fibres. Some "line-busy" effect should follow, therefore, for 0.5 to 1.5 second after a simple flexion reflex.

b. Electrical evidence of "line-busy" effect. In six successful preparations several hundred action currents in the peroneal nerve were studied. These comprised responses to direct stimuli applied at various intervals after

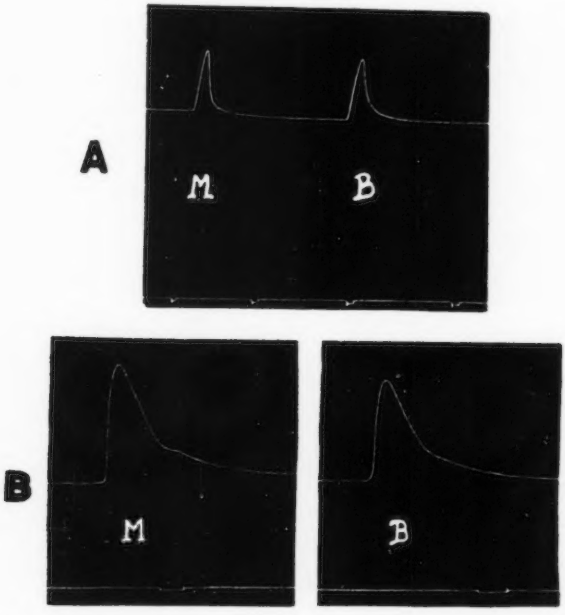

Fig. 2. Mechanical records made before severing motor nerve, to show duration of after-discharge with single stimuli; light lever used. A. October 21, 1927. Make and break shocks with drum moving continuously; time below in seconds. B. October 26, 1927. Make and break shocks with longer interval between recorded on faster drum, about 4 cm. per second.

reflex stimuli, and responses to two direct stimuli at various intervals. The first group of results may be considered first. Table 1 reproduces a complete series from each of two experiments, and table 2 summarizes all the results obtained on this point. The "line-busy" effect, as evidenced by a decreased response to a direct stimulus, is clearly no longer detectable when the reflex is evoked by a stimulus more than 50σ preceding the direct stimulus. The average duration of this maximum interval in all our experiments is 40σ. A typical set of records in an experiment of this sort is shown in figure 3.

TABLE 1

October 28, 1927. Cat, 4 hours after decerebration. Single maximal break shock to central end of popliteal nerve, followed by similar stimulus to peroneal. Observations made in the order given, about one minute apart. Action current led from peroneal nerve by boot electrodes to string galvanometer. String tension 320 M. per amp. November 3, 1927. Cat, 5½ hours after decerebration. Temperature in neck 35°, of isolated nerve about 30°. String tension 240 M. per amp. Height of reflex response to stimulation of popliteal, 0.4 mm. in all cases. Summary gives the average at each interval.

OCTOBER 28, 1927		NOVEMBER 3, 1927	
Interval between reflex and direct stimuli	Galvanometer excursion in response to the direct stimulus	Interval between reflex and direct stimuli	Galvanometer excursion in response to the direct stimulus
σ	*mm.*	σ	*mm.*
36	5.3	7.4	5.0
36	5.2	∞	5.1
36	5.3	7.4	4.9+
∞	5.4	11.3	4.8
32	5.3	11.3	4.6
32	5.3	∞	5.0
32	5.4	15.2	4.9
23	5.2	15.2	4.7
23	5.2	∞	4.9
23	5.3	19.2	4.9
∞	5.5	19.2	4.9
23	5.2	∞	5.0+
15	5.3	19.2	4.9
15	5.1	23.3	5.0
15	5.1	23.3	4.9
12	5.1	23.3	4.9
12	5.1	∞	5.0
12	5.1	31.8	4.9
∞	5.4	31.8	5.0
20	5.2	31.8	4.9
20	5.3	∞	5.0
28	5.2	31.8	4.9
28	5.2	46	5.0
∞	5.4	46	5.0
28	5.1	∞	5.0
32	5.2		
32	5.2	Summary	
36	5.3		
36	5.2	∞	5.0
36	5.3	7	5.0−
∞	5.4	11	4.7
		15	4.8
Summary: See table 4		19	4.9
		23	4.9+
		32	4.9+
		46	5.0

The interval between stimulating the popliteal and the arrival of reflex impulses under the electrodes on the peroneal should, of course, be subtracted from the above values to obtain the actual duration of discharge.

TABLE 2

DATE	CONDITION OF CAT	DURATION OF "LINE-BUSY" EFFECT
		σ
October 19	Decerebrate	35
October 26	Decerebrate	20
October 28	Decerebrate	>36
October 28	Cord transected 2 minutes before start	>45
November 1	Decerebrate	25
November 3	Decerebrate	50
November 9	Decerebrate de-afferented sciatic nerve	45
November 9	Dorsal roots intact	50

As determined by Forbes and Gregg (1915) for similar preparations, this correction is about 8σ (cf. also Forbes, Querido, Whitaker and Hurxthal, 1928). This is confirmed in the present experiments, as the direct response was maximum when the reflex stimulus preceded it by 8σ and was

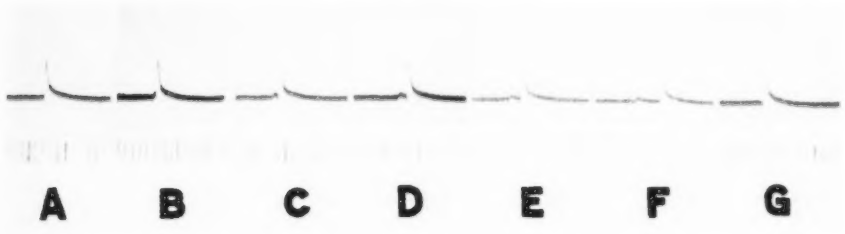

Fig. 3. Typical series of motor-nerve responses to show "line-busy" effect. Action currents of peroneal nerve recorded. Single stimulus applied to popliteal nerve (evoking flexion reflex) followed at various intervals by single stimulus applied directly to peroneal (motor) nerve.

November 3, 1927. Magnification in this and all other galvanometer records, 490. String tension, 240 M. per amp. A and G, motor-nerve stimulus alone; intervals in σ between reflex and motor-nerve stimuli in subsequent records:—B, 11.3; C, 15.2; D, 19.2; E, 23.3; F, 46. Time below in 0.01 second intervals.

The measurements of these excursions (motor-nerve stimuli) are included in table 1.

reduced most when the interval was increased to 13σ, that is, when the direct stimulus encountered the crest of the reflex activity. It is doubtful how far it is safe to go in quantitative interpretation of string displacements

where separate effects are being combined, so that the string is already in motion from the first when it begins responding to the second. (For constant currents, the displacement was shown to be accurately proportional to the current beyond the limits of these experiments.) Still it

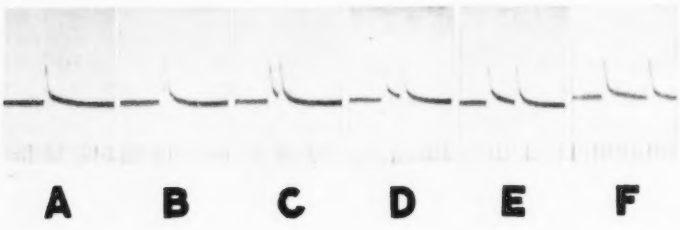

Fig. 4. Typical series of motor-nerve responses to pairs of stimuli, both applied directly to peroneal (motor) nerve. Each stimulus evokes a direct response followed by a reflex response which appears as a halting of the string in its return to the base line. The decreased height of the second excursion marks the extent of the "line-busy" effect; the decrease in the reflex portion of the second response, as compared with the first, marks the reflex decline. At the briefer stimulus intervals the second excursion is difficult to evaluate, since it is aided by the residual displacement of the string, but opposed by the inertia of its return. The "line-busy" effect is present in E, absent in F; the reflex decline is still present in F.

November 3, 1927. String tension, 240 M. per amp. A, single stimulus. Stimulus intervals in σ:—B, 7.4; C, 15.2; D, 23.3; E, 41; F, shows the 4th and 5th of a series delivered by hand; the interval between them is 60σ, but the first in the figure was preceded by a stimulus 170σ earlier, and therefore does not represent the resting condition.

is satisfying to note that the height of the direct response obtained at the crest of the reflex response is reduced by an amount equal to the height of the reflex response, and that the total displacement of the string is, therefore, that of a maximal direct response. The actual duration of the

Fig. 5. Typical records showing the decline of successive reflex responses; and correlation of decline with frequency. A and B, stimuli to popliteal nerve; C and D, stimuli to peroneal nerve. Leads on peroneal in all. A, November 9, 1927; deafferent limb, 5 hours after decerebration. String tension, 640 M. per amp. 1, rapid series of stimuli. 2 and 3, beginning and end of slow series; 2.25 seconds and one stimulus elapsed between end of 2 and beginning of 3. B, November 9, 1927; normal limb (dorsal roots intact), 7 hours after decerebration. String tension, 240 M. per amp. 1, fairly rapid series. 2 and 3, beginning and end of slow series; 2.24 seconds and two stimuli between 2 and 3. C, November 3, 1927. Reflex response is seen as hump on descending limb of motor-nerve response; greater on make than on break shocks. Stimuli delivered by rotary interrupter. String tension 600 M. per amp. D, November 9, 1927. Normal side, 7 hours after decerebration. String tension, 240 M. per amp. Reflex decline clearly seen in decreasing size of hump.

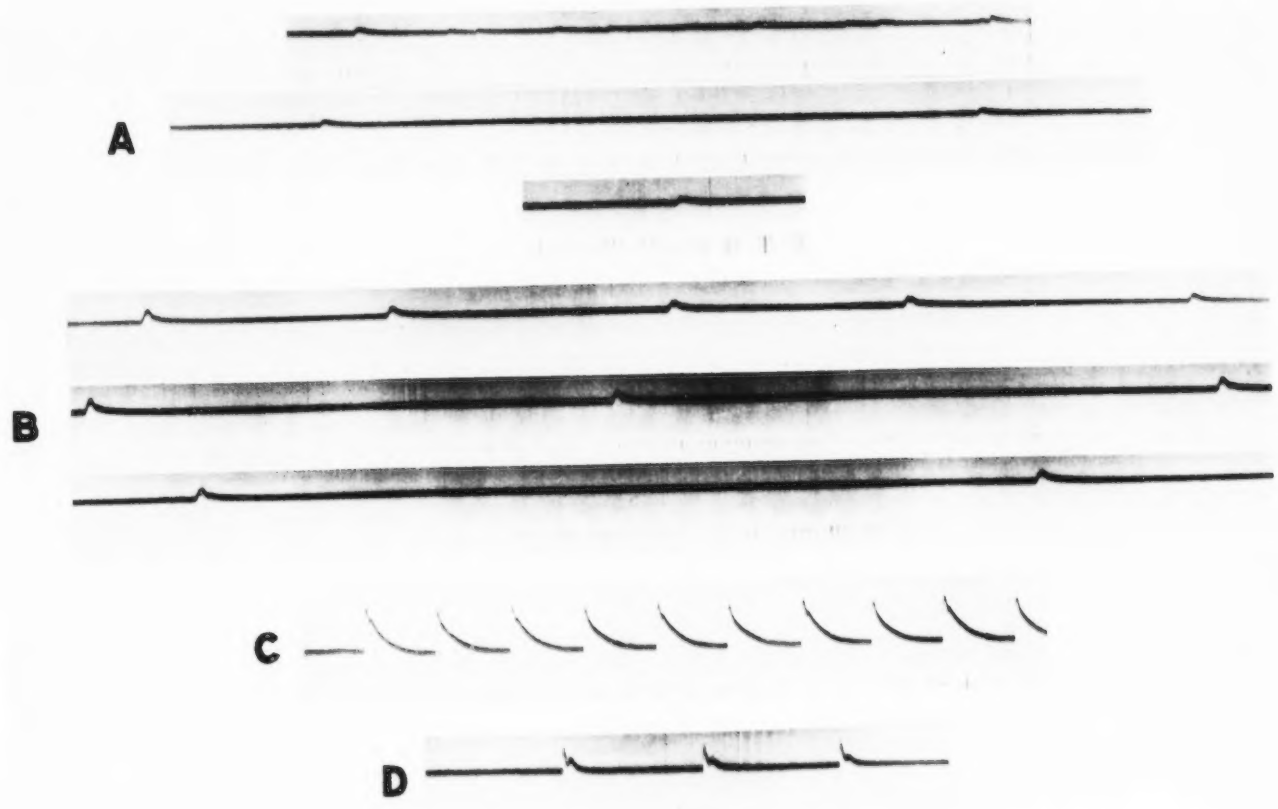

Fig. 5

"line-busy" effect, corrected for the period of transmission of the reflex, is then only about 30 to 40σ.

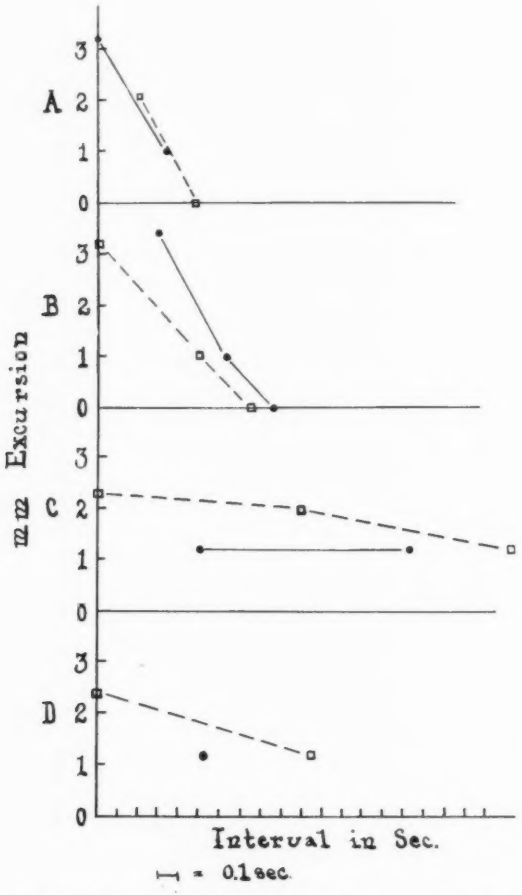

Fig. 6. Graphs showing decline of successive reflex responses at various frequencies of stimulation in experiment of November 1, 1927. Amplifier used; electric artefact renders uncertain the direct comparison of responses to make and break shocks; therefore makes and breaks are plotted in separate curves, but the time sequence of the stimuli is indicated. Abscissae, time in σ; ordinates, millimeter excursion on film. • = Make shock, □ = Break shock.

Similar results were obtained from experiments with two direct stimuli. The first stimulus gives an immediate maximal response of all fibres of the

peroneal nerve and a secondary reflex response of some of the motor fibres. A second stimulus will then illustrate two effects: a decreased height of the direct response, due to "line-busy" effect following the first reflex, and a decrease in the reflex response. If the reflex decline were entirely due to "line-busy" action, the second reflex should regain the height of the first at the same time interval that the second immediate response regains its initial value. In point of fact, the immediate response returns fully in 50σ or less, as in the other experiments, whereas the second reflex is still decreased at intervals greater than 300σ.

These experiments, though theoretically ideal to test the point in question, are subject to certain limitations. The height of the direct response undergoes changes with repeated rapid stimulation quite aside from any reflex effects (see the preceding paper), and also under the conditions of our experiments, at the shorter intervals, the string was still considerably deflected from the first action current when the second

TABLE 3

In each test the stimuli were repeated at the constant interval indicated. Resting value is the initial size of response after a rest of at least 2 seconds. Equilibrium value is the size the responses reached when they ceased to decline further; equilibrium was reached between the 3rd and the 10th response.

INTERVAL	RESTING VALUE	EQUILIBRIUM VALUE
second	*mm.*	*mm.*
0.1	1.4	0.5
0.12	1.7	0.6
0.25	1.1	0.7
0.4	1.6	0.9
0.8	1.5	1.2

one began. Figure 4 reproduces records from one of the experiments which gave clearcut results relatively free from the errors just considered, and illustrating the disappearance of "line-busy" effect when the decreased reflex response is still marked.

c. Diminution of successive reflexes. Besides the observations just described, a number of experiments were made to determine more exactly than has previously been done the conditions of decline of the reflex response on repetition. A large number of observations were made of action currents in the motor nerve in response to repeated reflex stimulation by hand or with the rotary interrupter. In several of these experiments the amplifier was used. A typical set of records is shown in figure 5. The detailed results will not be reported, for similar experiments have been described previously, but the data in figure 6 and table 3 illustrate the findings. In the same experiment that furnished the data of table 3 we observed the return of the reflex from the low level of repeated stimu-

lation towards that of rest when stimuli were sufficiently separated. This was done by running the rotary interrupter at gradually decreasing speed, and thus progressively increasing the interval between stimuli. In one instance an interval of 1.3 second sufficed for recovery of the response from 0.9 mm. to 1.3 mm. The series shown in figure 6 is rather unusual in that the reflex fades out completely in two cases; in most cases even with rapid stimulation some constant positive value is maintained.

In one experiment several tests were made in which the peroneal nerve (still connected with the center) was stimulated by means of the rotary interrupter. In some of these the speed of the interrupter was constant, and the decline of the reflex could be seen in the decrease in size of the notch in the descending limb of the record. The decline is less in this case than in the popliteal-peroneal reflex, but it is still apparent (fig. 5, C). In one case the interrupter was started at slow speed, delivering impulses at intervals of about 450σ, and then gradually accelerated till the intervals were reduced to 37σ. When this was done the reflex notch decreased progressively more and more, as with decreasing intervals the level of equilibration became less.

In one experiment in the present series and several others performed previously in another connection by one of us (A. F.), two reflex stimuli to the popliteal were delivered at short known intervals by means of the Lucas pendulum. In these experiments the second response was smaller than the first at all intervals, as usual, but when the stimulus interval was between 20 and 30σ the second response was greater than at briefer or longer intervals. The exact significance of this is not clear, though possibly related to central summation. It is more fully discussed elsewhere (Forbes, Querido, Whitaker and Hurxthal, 1928) and in any event does not seriously affect the considerations for longer intervals.

These experiments indicate that when reflexes are evoked at intervals of 0.2 second or less there is a marked and progressive fall of response during the first 3 to 5 reflexes to a third or less of the original value, and this new level is maintained while stimulation at the same interval is continued. At 0.8 second intervals the fall is slight from the first to the second response and absent after this. If stimuli at 0.8 second intervals are begun when the reflex is at a low level from previous more frequent stimulation, some return to a higher level may occur. At longer intervals definite fading has sometimes been observed, but recovery of an already diminished reflex will easily take place. Our results do not justify a more quantitative statement than this, which, however, is sufficient for the point in question. It is obvious that the decrease of the flexion reflex on repetition, as measured by action currents, persists long after the "line-busy" effect, measured in a similar manner, is no longer detectable.

d. Interrelation of flexion reflexes. The procedures followed for the

above determinations allowed, incidentally, observations on the effects of a reflex elicited from the popliteal nerve on one from the peroneal, and vice versa. We wish to record only one consistent phenomenon, already noted under different conditions by Forbes (1912), namely, the direct peroneal reflex is relatively little affected by preceding reflexes via the popliteal. This is in contrast to the decline in this reflex on repetition. The difference is illustrated in figure 7. Probably the same is true in the reverse direction—preceding peroneal reflexes have little effect on popliteal ones—though we have few observations on this point. This will be considered in connection with other facts to follow.

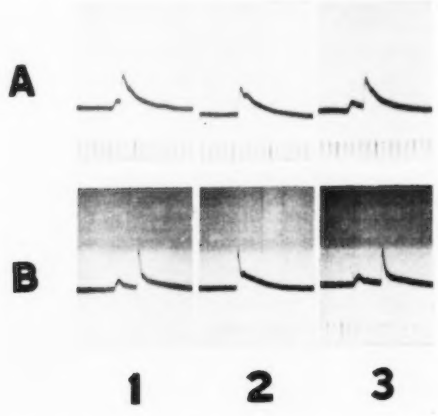

Fig. 7. Records showing effect of reflex evoked through popliteal nerve on subsequent reflex evoked through peroneal. In 1 and 3 of each row the peroneal stimulus is preceded by a stimulus to the popliteal nerve; in 2 only the peroneal was stimulated. The reflex response to peroneal stimulus (the hump on descending limb of curve) is only slightly reduced by previous reflex from the other nerve. A, October 19, 1927. String tension, 480 M. per amp. Stimulus intervals: 1, 17σ; 3, 27σ. B, November 9, 1927; normal side (dorsal roots intact). String tension, 240 M. per amp. Stimulus intervals: 1, 46σ; 3, 52σ.

e. Synchronism of discharge. The galvanometer string, of course, records the sum of all electric changes occurring at once in the nerve. When the fibres are all responding in phase their action currents approximately coincide and produce the maximum deflections of the string. As they become more and more asynchronous the action currents from various fibres conflict. If the leads are diphasic no response whatever should result with entirely asynchronous impulses, and even with monophasic leads such activity would only result in a small, sustained shift of the base-line which would be hardly detectable.

The motor discharge of the popliteal reflex, even at its height, is not entirely in phase. This is indicated by the rise and especially the fall of the deflection being slower and the summit more rounded than in the case of a direct stimulus to the peroneal nerve (see also Forbes and Gregg, 1915). Also, when diphasic leads are used, the reflex response is almost entirely smothered.

In contrast to this, the reflex evoked from the peroneal nerve itself appears to involve a much more simultaneous discharge. The reflex "notch" on the descending limb of the direct response is very sharp, with an abrupt rise and fall (see fig. 5 C, and 7), and it is not obliterated by making the leads diphasic (cf. Forbes and Gregg, 1915, fig. 8).

In several experiments peroneal action currents were regularly double in reflex response to single shocks applied to the peroneal or popliteal or (in one experiment) the anterior crural. Such responses are shown in figure 8 and are reminiscent of those described by Forbes and Gregg (1915).

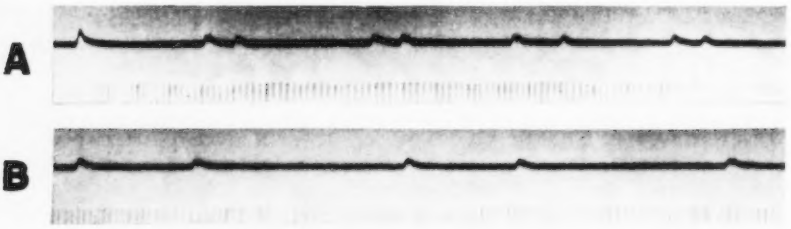

Fig. 8. Records showing double-peaked reflex responses. Stimuli applied to popliteal nerve. The double peak becomes more marked as the size of response declines.

They would indicate either separate fibres responding in two groups, or a distinct double volley in many of the fibres.

f. The influence of spinal transection. Transection of the cord was performed in three experiments, but in only one were adequate series of observations made before and after this procedure. As previously reported (Forbes, Cobb and Cattell, 1923) spinal section was followed by an increased reflex response. The "line-busy" effect was essentially the same before and after transection, as illustrated in table 4.

g. The influence of de-afferentation of the sciatic. In one cat with the dorsal roots of the sciatic nerve cut 12 days previously, complete records were obtained from both normal and operated sides, using the anterior crural nerve for reflex stimulation in both cases. The scatter of individual observations on the "line-busy" effect was rather large so that accurate comparison is not possible, but no outstanding differences are apparent. Table 5 contains the averages. The experiment serves to demonstrate,

at least, that the "line-busy" effect studied is not dependent on intact proprioceptive arcs, and also that the reflex effect of the direct stimulus does not interfere with the interpretation of results in the intact preparation.

TABLE 4

October 28, 1927. See table 1. Column 3 from data obtained 3 to 15 minutes after cord severed.

In both cases the "line-busy" effect is present at intervals of 36σ.

INTERVAL BETWEEN STIMULI	SIZE OF DIRECT RESPONSE	
	Before transection	After transection
σ	*mm.*	*mm.*
12	5.1	
15	5.2−	5.0−
20	5.2+	5.1
23	5.2+	5.2−
28	5.2−	5.1+
32	5.3+	5.2−
36	5.3−	5.2+
∞	5.4+	5.3+

TABLE 5

Comparison of "line-busy" effect in de-afferent limb and in limb with dorsal roots intact. Successive groups of observations at different string tensions shown in separate columns in chronological order. In each group all measurements at a given stimulus interval are averaged.

String tension in M. per ampere: a. 320, b. 640, c. 240, d. 240, e. 640.

STIMULUS INTERVAL	EXCURSION IN RESPONSE TO DIRECT STIMULUS				
	De-afferent side			Intact side	
	a	b	c	d	e
σ	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
∞	3.9	4.2	3.7	5.1	5.5
7.4	3.9	4.2	3.7	5.1	5.3
11.3				5.1	5.5
15.2	3.7	4.1	3.4	4.7	
19.2					5.2
23.3	3.7	4.1	3.6	5.0	
31.8	3.7	4.1	3.4	4.9	5.1
46.0	3.9	4.1	3.6	4.9	5.2

Similarly, the typical diminution of the reflex on repetition was observed on both sides.

h. Miscellaneous observations. When the peroneal nerve is stimulated by a single shock, both the direct and reflex responses appear in the same record but may be separately measured. It is thus very simple to com-

pare the direct and reflex effects of various strengths or kinds of stimuli. Such a comparison shows at once that the two do not vary concomitantly, though the divergence is not constant. It has appeared in many but not all cases that the make shock is more effective than, or equally effective with, the break in eliciting a reflex, whereas the break was always more effective than the make in evoking the direct response. This would be in harmony with the observations of Erlanger (1927) that the more slowly conducting fibres are sensory.

Discussion. The results obtained seem to demonstrate positively the existence of a "line-busy" effect following a single flexion reflex, lasting about 40σ. The decline in successive reflexes, on the other hand, is still demonstrable when these are elicited at intervals of 0.8 second or more. The "line-busy" effect is, therefore, inadequate as a full explanation of the reflex decline, and some other factor or factors must play a rôle in its production. One such factor may well be that of "equilibration" previously discussed. In the case of frog's peripheral nerve, the size of response falls, on continued tetanization, to less than one-third the initial value, but the fall is not manifested during a few impulses, as in the case of the reflex, but in the course of thousands. In peripheral nerve the rate of decline of response with activity varies with the intensity of the activity. Central nervous tissue exhibits an oxygen consumption about 70 times that of the nerve fibres (cf. Loebel, 1925, and Gerard, 1927b), and as only a small fraction of the central tissue is composed of nerve cells, the rate for these may be much greater. It is quite conceivable, therefore, that "equilibration" in the reflex arc should manifest itself with much greater rapidity than in peripheral nerve. Whether, in fact, the decline of reflexes is essentially the same phenomenon as in nervé fibres and, therefore, to be given this specific term cannot be decided on present evidence; but some form of "fatigue" is obviously suggested and the simplest assumption for further work is that of identity in the fibre and cell.

Since the nerve fibre acts in all-or-nothing fashion, any change in peroneal action currents must represent changes in the number of active motor neurones or in the frequency of discharge of those active. A decreased number of active cells and a decreased discharge per cell may both be concerned in the decline of successive reflexes.

The first factor would represent complete refractoriness of some cells for varying periods after activity. Thus after a single reflex discharge say 50 per cent of the cells would be refractory for 0.1 second and a second reflex at this time would produce an action current $\frac{1}{2}$ the original size. In 0.5 second only 25 per cent would still be refractory and in one second none, so that second responses at these intervals would be respectively $\frac{3}{4}$ and full sized. This demands a statistical variation in the refractory periods of separate cells, which is not in itself improbable. But some of

the reflex units, at least, have refractory periods of only a few sigmata (Cooper and Adrian, 1924; Forbes, Barbeau and Rice, 1927). It seems highly improbable that a good fraction of the units involved in the flexion reflex have very short refractory periods and the remainder are refractory for from 0.1 to 1.0 second. Also, this interpretation meets difficulty in accounting for a further fall of the reflex from the second to the third response. If the same interval elapsed between the second and third as between the first and second stimuli, the same neurones that were able to respond to the second should be able to respond to the third stimulus, and in addition some of those that were refractory to the second should have recovered during the added interval and be again excitable by the third stimulus. The third response should thus be greater than the second, whereas it is always equal or less. An explanation based solely on fixed refractory periods, therefore, is not possible. But one manifestation, if not the most important one, of equilibration in nerve fibres is a progressive increase in the refractory period with repeated activity, and a similar change in the nerve cells might play a rôle in the progressive fall of the reflex.

Probably a decreased discharge by each motoneurone plays a larger rôle than the complete elimination of some in reducing the motor discharge. Sherrington (1921) and Fulton and Liddell (see Fulton, 1926, p. 292; cf. Adrian and Forbes, 1922, p. 323) have shown that the muscle twitch evoked by a single shock to an afferent nerve, producing a flexion reflex, is usually a short tetanus at high frequency. Six volleys at 200 a second would thus correspond with the 30σ duration of the "line-busy" effect without any further after-discharge. The deflection of a string galvanometer, however, does not show any periodicity, as the individual action currents are more or less fully summed. Any increase or decrease in the number of volleys evoked by a stimulus would thus be shown by a similar change in the electrical record, and "equilibration" in the reflex arc could manifest itself as a progressive decrease in the number of volleys in response to a single stimulus.

The increased action current and muscle response following transection of the spinal cord appear similarly to be the effects of an increased number of immediate motor volleys in the spinal, as compared with the decerebrate, preparation (Sherrington, 1921; Sassa and Sherrington, 1921). The nature of this "release" remains unknown. The two effects observed by Forbes, Cobb and Cattell (1923), viz., an absolute increase in the single reflex response and the lessened decline on repetition, may both be manifestations of the same release; since whatever factor causes the motor volleys to increase in number might well cause them to maintain themselves better with repetition. The original interpretation of this as lessened "line-busy" effect due to cutting delay paths, and so reducing

after-discharge, seems inadequate, since the "line-busy" effect does not account for much of the fall in any case and shows no significant difference after spinal section. Experiments on newborn rats carried out by one of us (R.W.G.) also show an immediate and marked increase of reflex activity of the rear part of the animal upon section of the cord in the thoracic region. The work of Ballif, Fulton and Liddell (1925) and others also throws doubt on the view that delay paths are responsible for long after-discharge. Destruction of delay paths of inhibiting as well as excitatory effects might be expected on spinal section, so that on this interpretation both should be shortened. In fact, however, the after-discharge of excitatory reflexes is usually much reduced by section of the cord, but that of inhibitory reflexes, as inhibition of the knee jerk, is equally augmented.

In closing we wish to consider briefly the question of reflex interaction. Neither the reflex from the peroneal nerve nor that from the popliteal reaches all the motor arcs of the peroneal, but Cooper, Denny-Brown and Sherrington (1926) report that the popliteal may excite 75 per cent, and the musculo-cutaneous branch of the peroneal 70 per cent of all the motoneurones. There must be, then, an overlap of afferent connections from these two nerves on at least a third of the motoneurones reached, and possibly a much greater number. The fact that a reflex evoked from one nerve may not decrease a subsequent one evoked from the other would thus suggest that the effects are produced up-stream from the motoneurone itself (cf. Forbes, 1912). The greater synchronism of discharge of the direct peroneal reflex than that of the popliteal also suggests intervening mechanisms between the afferent nerves and the motor cells, there being more in the case of the less direct reflex. It is profitless to speculate on the rôle of synapse and cell body and the number of neurones in the various arcs till more information is available. In all the above discussion the term "cell-body" is used loosely to include any of the extra-axonic structures in the central nervous system.

SUMMARY

1. A method is described for measuring the "line-busy" effect following a flexion reflex. A maximal direct stimulus along the course of the motor nerve will produce a smaller action current when some of the fibres are occupied by continued reflex activity than when all are at rest. Flexion reflexes were produced by single maximal stimuli to the peroneal or popliteal nerve and the motor-nerve action currents led from the ipsilateral peroneal.

2. Although mechanical records of the flexion reflex evoked by a single stimulus show a small amount of after-discharge for 1 to 4 seconds, the "line-busy" effect in either the decerebrate or low spinal preparation is not detectable by this method more than 30 to 40σ after the start of the reflex response.

3. The electric responses of successive reflexes from the popliteal stimulus are progressively diminished if elicited at intervals less than 0.8 second and perhaps longer intervals as well; the shorter the intervals the greater is the diminution, but complete extinction is rare. A minimum of diminution sometimes appears at intervals of 20 to 30σ. Similar results are obtained with reflexes elicited from the peroneal nerve, but a reflex from the popliteal had little effect on a subsequent one from the peroneal.

4. The motor discharge in the peroneal-peroneal reflex is more synchronous than that in the popliteal-peroneal reflex, though both may give double-peaked action currents.

5. From these results it is concluded that the progressive decline in reflex response on repetition is due only in small part to the "line-busy" effect. Another factor or factors must play a rôle, and a fatigue effect analogous to that of "equilibration" in peripheral nerve is suggested.

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ELECTRON EQUILIBRIA IN BIOLOGICAL SYSTEMS

II. The Simultaneous Automatic Recording of Electrical Potential and Muscular Contraction

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Under the general title "Electron Equilibria in Biological Systems" a preliminary report was published in the Public Health Reports (1). At that time a brief survey of the factors leading to the development of a satisfactory method for the study of electrical potential changes in tissues was given. The construction of a two-stage thermionic vacuum tube amplifier was described, and its usefulness for the measurement of potentials in biological systems pointed out. Since the publication of the preliminary report, a continuation of the studies on smooth muscle structures, together with an extension of the investigation to skeletal and cardiac muscle has necessitated the improvement of the equipment formerly used and the development of more satisfactory methods for the recording of results. It is the object of this paper to present a full description and discussion of the equipment in use at the present time.

Methods. For the purpose of completeness it is desirable at this time to include a diagram of the thermionic vacuum tube amplifier. Certain alterations have been made in the diagram in an effort to make more evident the operation of the equipment and the function of the potentiometer. The only modification of the apparatus has been its complete shielding, the substitution of a twenty-four volt storage "C" battery, and a ninety-six volt storage "B" battery for the twenty-two and a half volt and ninety volt dry batteries previously employed. A two-volt storage cell has been substituted for the two dry cells on the potentiometer. These changes have been indicated in figure 1.

The first stage of the amplifier is supplied with a UX 240 tube, because of its high amplification, while the second stage is equipped with a UV 201A. The plate or output circuit of the UX 240 tube feeds into the grid circuit of the UV 201A tube through a variable $\frac{1}{2}$ meg ohm resistance connected in series with a 24 volt C battery, the variable resistance acting as one of the controls for adjustment to maximum sensitivity. The milli-

voltmeter in the plate circuit of the UV 201A tube is shunted by a 3,000 ohm variable resistance which acts as the second control for adjustment of maximum sensitivity.

By proper manipulation of the variable shunt resistance, R_3 and the two single-pole double-throw switches *1* and *2*, it is possible to adjust the apparatus without damage to the millivoltmeter. With switches *1* and *2* in the "down" position and all the resistance in R_3 cut out, the filament current is turned on. Switch *2* is now thrown to the "up" position

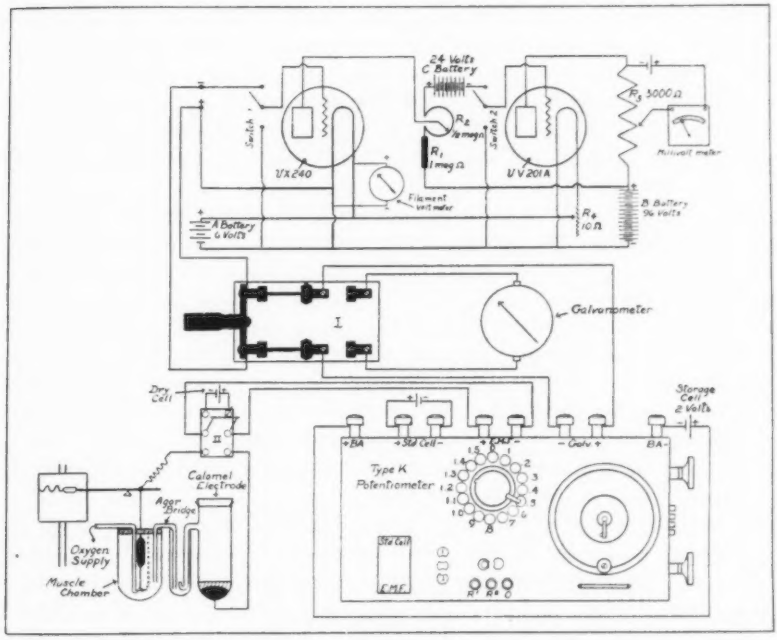

Fig. 1

and the desired zero point of the millivoltmeter adjusted by means of R_2 and R_3. Then switch *1* is thrown to the "up" position and the adjustment of R_2 and R_3 repeated, the sensitivity being greater the larger the amount of resistance used in the shunt R_3. The best results have been obtained with a filament voltage of 5.1–5.2, B battery voltage of 96, and C battery voltage of 24.

An inspection of the diagram makes it evident that if the large double-pole double-throw switch *I* is thrown over to the right so as to make connections with the galvanometer to the right of the switch, and the switch

II is thrown in the "down" position, and if a hydrogen electrode and vessel is substituted for the muscle chamber we have the usual equipment for the measurement of hydrogen ion concentration. If now the switch *I* is thrown to the left the amplifier is put into the circuit and we have essentially the equipment of Goode (2), which may be used for the continuous measurement of changes in hydrogen-ion concentration. In both cases the function of the potentiometer is the same. The recording millivoltmeter may be used as a null-point instrument and the voltage read off directly on the potentiometer. However, for reasons to be given later, it is necessary to know the value of a scale division deflection on the millivoltmeter and therefore provision has been made for establishing connections with a dry cell by throwing switch *II* to the "up" position. The potentiometer is adjusted so that the millivoltmeter reads zero. By moving the potentiometer drum so as to cause a deflection of the needle it is possible to read off the voltage necessary to produce the deflection, thus permitting calibration of the millivoltmeter scale.

At this point, a bit of evidence may be given as regards the reliability of the equipment. The voltage of the calibrating dry cell was determined by two methods. With switch *II* in the "up" position and switch *I* thrown to the left, the millivoltmeter was used as a null-point instrument and the potentiometer showed the voltage of the dry cell to be 1.2347. With switch *I* thrown to the right the amplifier was cut out of the circuit, the usual potentiometer equipment was being used and the voltage of the dry cell was found to be 1.2345, a difference of 0.2 millivolt between the two methods. Inasmuch as withdrawal of current from the dry cell is eliminated when the amplifier is in the circuit it seems reasonable to conclude that the value 1.2347 is the more accurate.

Lastly, it is emphasized that *the potentiometer is connected in series between the grid of the first tube and the electrode in the tissue.* Under these conditions the charge upon the grid of the first tube is negative with reference to the filament and prevents current flow in the grid circuit. This is a circumstance of the greatest importance for the measurement of potentials in biological systems, for it meets the requirement of measuring potential variations without the withdrawal of current from the systems of small capacity under investigation. Withdrawal of current would of course change the physico-chemical condition of the tissue.

When the equipment is in operation it is essential that drift of the millivoltmeter needle, due to causes extraneous to the source of potential being measured, be reduced to a minimum. The causes of such drift are changes in temperature of the resistances, particularly those in the filament circuit of the tubes, change in voltage of the battery supplying the filament current and change in voltage of the B and C batteries. The temperature effects are negligible providing the room temperature is constant within one degree

over a one-hour period. The use of storage B and C batteries, kept properly charged, eliminates the difficulty caused by the use of dry cell B and C batteries. The use of a large capacity, 150 ampere hour storage battery for the filament current removes error from that source, providing the battery is used so that it operates on the flat part of the discharge curve. If the battery has been recently charged it will be necessary to light the tubes long enough before one experiment to permit a steady zero point.

There is no qualitative difference between the measurements made with the equipment described and those made with the usual potentiometer apparatus. *The character of the measurements is dependent upon the character of the electrodes employed.* If a calomel-half cell and a hydrogen electrode are used, the values obtained are a measure of hydrogen-ion concentration, providing a hydrogen-ion buffer system is present; if a calomel-half cell and a blank platinum electrode are used, the values are a measure of oxidation-reduction potential providing an oxidation-reduction poising system is present; if two calomel-half cells, or two non-polarizable electrodes are used, the values are a measure of the potential difference between the electrodes. But since the apparatus draws no current from the source being measured, it is logical to conclude that when a calomel-half cell and a hydrogen electrode are being used in systems poorly poised with respect to hydrogen-ion concentration, and when a calomel-half cell and a blank platinum electrode are used, systems poorly poised with respect to oxidants and reductants may be measured more accurately than with the ordinary potentiometer equipment.

Therefore in our studies of biological systems employing a calomel-half cell and a blank platinum electrode, the values are not a measure of changes in conductance, for no current flows; they are not a measure of changes in potential difference pure and simple, they are not a direct measure of alterations in hydrogen-ion concentration, but they are a measure of changes in oxidation-reduction potential influenced, of course, by other factors. One of these factors which is definitely known is changes in hydrogen-ion concentration, for oxidation-reduction potential is a function of pH and vice versa. Biological systems are characterized by membranes, large surface areas, and phase boundaries. Since changes in oxidation-reduction potential involve electron transfer, it is necessary to assume, until proved to the contrary, that all processes involving electron transfer, such as changes in membrane potentials, adsorption potentials, diffusion potentials, etc., as well as changes in hydrogen-ion concentration, may alter oxidation-reduction potentials in living systems. Because of these complicating factors it seems best, for the present at least, to speak of "electron equilibria" rather than of oxidation-reduction potentials in biological systems.

In the work previously reported we were dealing with slowly and rhyth-

mically contracting smooth muscle structures for the most part. For that reason a recording instrument having a high frequency of vibration was not necessary. A millivoltmeter having a resistance of 25 ohms and capable of giving a perceptible deflection with one volt acting through a resistance of 600,000 ohms served our purpose. The scale divisions of this instrument were calibrated in terms of millivolts as described earlier in the paper. Having determined, for example, that with a given adjustment of the controls on the amplifier each scale division of the millivoltmeter is equivalent to one millivolt as indicated directly by the potentiometer reading, it is possible to use the apparatus in two ways. For slowly contracting tissues such as the guinea pig or rabbit uterus, the millivoltmeter may be used as a null-point instrument and the voltage changes accompanying contraction and relaxation may be continuously followed by rotating the potentiometer drum. It is possible in this manner to correlate the voltage values and the phase of uterine contraction with a surprising degree of accuracy. When, however, studies were made on more rapidly contracting tissues such as a segment of rabbit duodenum, a turtle heart *in situ*, or a turtle ventricular strip, it was impossible to follow the voltage changes by continuous adjustment of the potentiometer. Instead the potentiometer was adjusted to a satisfactory basic value about which the voltage fluctuated and the alterations were read off directly on the millivoltmeter scale, one division being equal to a millivolt.[1] Under these conditions, it was easy to observe the maximum and minimum voltages and correlate them with the trough of relaxation and the peak of contraction respectively, but it was impossible to follow changes in greater detail.

To correlate the voltage changes and the phase of contraction more accurately in the case of rapidly contracting tissues, other methods of recording were employed. An oscillograph, part of an electrical telemeter designed by Burton McCollum and O. S. Peters of the Bureau of Standards, was used (3). In brief, the instrument provides for the automatic registration on a moving photographic film of the movements of a mirror attached to the string of the specially constructed string galvanometer. The reader is referred to the literature for a detailed description of the telemeter. The particular instrument used provides for the simultaneous registration of as many as twelve strings.

The oscillograph was connected in series with the millivoltmeter in the output circuit of the thermionic-vacuum tube amplifier. This arrangement permitted observation of the voltage changes simultaneously with the taking of a record on the moving film of the oscillograph, making it possible

[1] The fact that direct readings from the millivoltmeter with stationary basic value of the potentiometer check readings obtained by continuous adjustment of the potentiometer, the millivoltmeter being used as a null-point instrument, furnishes further evidence that no current is drawn from the tissue.

to keep the potentiometer so adjusted that the beam of light registering voltage changes remained on the film. Three of the strings having a frequency of approximately 300 cycles per second were used. One string was used to register time. For slow speeds of film the string was made to record seconds by being connected in series with a Harvard electrical time clock through a resistance of 10,000 ohms, and for rapid speeds of film the string was placed in series with 5,000 ohms and an electrically recording tuning fork of fifty vibrations per second operating on one dry cell. Another string connected in series with the millivoltmeter as described above recorded the voltage changes. A third string recorded the muscle contraction by means of the arrangement shown in figure 2.

A glass tube having an internal diameter of at least 2.5 cm. and a length of 20 cm. is closed at the lower end with a rubber stopper provided with a

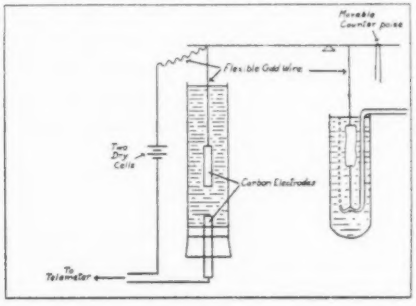

Fig. 2

carbon electrode. The tube is clamped in a vertical position and the fixed carbon electrode connected to one terminal of an oscillograph string. A movable carbon electrode is suspended from the end of a light writing lever by a fine flexible gold wire which also serves to make electrical connection with one terminal of two dry cells connected in series, the other terminal of the dry cells connecting the second terminal of the oscillograph string. The tube is filled with distilled water to which a small amount of sodium chloride is added. Movements of the muscle produce corresponding changes in the position of the movable carbon electrode and the changes in resistance between the electrodes cause deflections in the oscillograph string. By adjustment of the amount of salt added to the tube and the proximity of the electrodes, the magnitude of the string deflection and the position of the reflected beam of light on the film may be controlled. The lever is provided with a movable counterpoise. When working with a frog gastrocnemius muscle in situ or suspended in a moist chamber, the lever may be dispensed with, the movable carbon electrode being directly

connected by a silk thread with the lower end of the muscle preparation. Graduation marks may be made on the glass tube and the deflection of the oscillograph string calibrated in terms of the degree of displacement of the movable electrode.

Using the technique described, it becomes possible to automatically and simultaneously record time, muscle contraction, and voltage change on the same photographic film.

The method of preparing and of inserting the electrode into the tissues,[2] the composition of the saline solutions and other technical details were the same as described in the previous publication. Sufficient time was allowed for the tissue to adjust itself to the environment, after which rhythmic contractions and potential changes showed a good correlation for a long time.

For the electrical stimulation of nerves innervating the muscles under study, currents were used which were just sufficient in strength to produce a marked effect.

RESULTS. The methods here described have been applied to studies on guinea pig and rabbit uterus, rabbit duodenal segments, turtle heart *in situ*, turtle ventricular strip, and frog gastrocnemius muscle *in situ*.

In the tracings the bottom string records the time and serves as a base line; the middle string records the muscle contraction (upstroke); the third string records the voltage changes, downstroke indicating a decrease and *vice versa*.

In reproduction figures 3 to 10 inclusive were reduced to one third their original size. The voltage changes per millimeter as given in the legends must therefore be multiplied by three in order to obtain the actual values.

The relationship of voltage change to phase of contraction previously reported has been confirmed. The records obtained have indisputably established the fact that contraction is accompanied by a sharp fall in potential and that relaxation is characterized by a building up in potential.

There can, therefore, be no question that the chemical changes responsible for the potential changes are intimately connected with the contraction and relaxation of the various types of muscles. With rhythmically contracting smooth and cardiac muscle the cycle of potential change is completed with the completion of each single contraction and relaxation. In the case of the frog gastrocnemius (fig. 9), however, the building up of voltage still proceeds after complete relaxation. The reason for this is not evident at present. It should also be noted that in this case the smaller oscillations in the muscle curve were caused by spring in the light lever used and, what is important, these smaller oscillations were not accompanied by corresponding voltage changes.

Faradic stimulation of the vagus nerve (fig. 8) caused a pronounced in-

[2] Particular care must be exercised to insure that the blank electrode is actually *inside* the tissue and that it is not in direct contact with the saline solution.

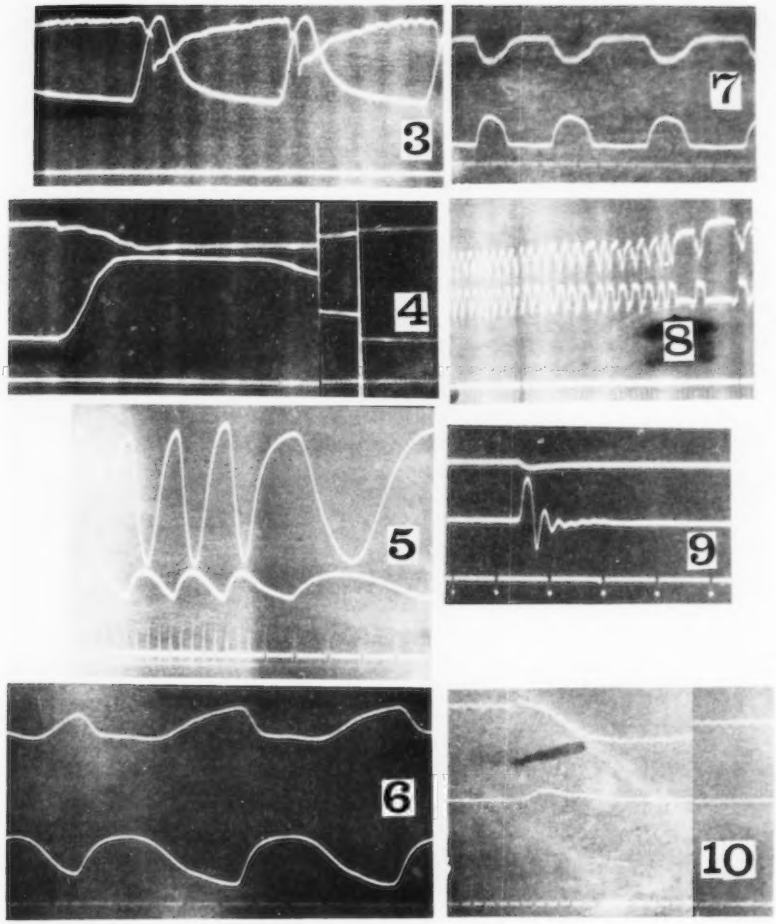

Fig. 3. Non-pregnant guinea pig uterus. Time = seconds. Basic voltage in the relaxed state 771 m.v.; 1 mm. displacement of the beam = 1.68 m.v.

Fig. 4. Same muscle as in figure 3 with faster film. Time = seconds. Basic voltage = 737 m.v.; 1 mm. = 1.65 m.v. Interval between parts 1 and 2 = 30 seconds, between 2 and 3 = 90 seconds.

Fig. 5. Rabbit duodenal segment. Two different speeds of film. Time = seconds. Basic voltage = 552 m.v.; 1 mm. = 1.9 m.v.

Fig. 6. Rabbit duodenal segment. Faster film than in figure 5. Time = seconds. Basic voltage 324 m.v.; 1 mm. = 1 m.v.

Fig. 7. Turtle ventricular strip. Time = seconds. Basic voltage = 353 m.v.; 1 mm. = 1 m.v.

Fig. 8. Turtle heart in situ. Effect of vagus stimulation on ventricle. Vagus stimulation begun at black line on time record. Time = seconds. Basic voltage = 745 m.v.; 1 mm. = 1 m.v.

Fig. 9. Isolated frog gastrocnemius in moist chamber. Stimulation of sciatic nerve by single break shock. Time = seconds. Basic voltage = 735 m.v.; 1 mm. = 1.65 m.v.

Fig. 10. Frog gastrocnemius *in situ*. Faradic stimulation of peripheral end of cut sciatic by single break shock. Secondary soil at g.cm.; load 2 g. Time = $\frac{1}{50}$ second. Basic voltage 777 m.v.; 1 mm. = 1 m.v. Interval between part 1 and 2, 1 second. Small waves on potential curves are result of 60 cycle alternating house current and were caused by inadequate shielding.

crease in the voltage during the period of cardiac inhibition, a fact which may indicate the storage of potential chemical energy as a result of decreased functional activity.

The interpretation of the chemical significance of the potential changes will be discussed in a subsequent paper on the basis of experimental evidence which has been accumulated.

SUMMARY AND CONCLUSIONS

1. Improvements in the electrical equipment for measuring voltage changes in contracting tissues have been described, and the significance of the values obtained has been briefly discussed.

2. Methods for the continuous automatic and simultaneous registration of muscle contraction and potential changes have been presented.

3. The results previously reported on contractile tissue have been extended and confirmed with the more exact technique.

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FACTORS WHICH DETERMINE RENAL WEIGHT[1]

IV. Pregnancy and Lactation

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It is generally believed that pregnancy causes an increase in the size of some of the organs, notably the liver and kidneys. In the rat there is a true growth stimulus during pregnancy and aside from the obvious temporary increase in body weight there is a permanent increase in weight associated with and continuing after the period of pregnancy (Watson, 1905; Hartwell, 1927). It is not known whether this increase in body weight is due to a general and uniform increase in all the tissues and organs or may perhaps be mainly due to an increase in a particular tissue like fat or in some particular organ system (such as the liver, pancreas and kidneys, —e.g., a glandular increase). At any rate, whether general or local, here is a physiological condition which influences growth. The question is whether the condition of pregnancy has any specific effect on the kidney which is not observed on other glands, such as the liver, or other organs, such as the heart. In an attempt to answer this question the effect of pregnancy on the weight of the kidneys and, for the purpose of comparison, on the weight of the heart and the liver has been determined.

The effect of pregnancy in young adult rats—(Expt. 1). Female rats 166 days of age were used for this experiment. At this time they are sexually as well as physically mature and at an optimal age for bearing young. The general methods which were used have already been described (MacKay and MacKay, 1927). In this and the other experiments reported here the experimental female diet which contains 10 per cent of fresh wheat germ was used. The latter insures the adequacy of the food for breeding and lactating rats in so far as the fertility factor (Evans and Bishop, 1923) is concerned. The control group of rats for this pregnancy experiment consisted of an unselected group which were placed upon the special diet when 166 days of age. These were killed 54 days later, or when they were 220 days of age. The mean daily food intake and body weight of the group during the period of observation are charted in figure 1. In table 1 are the

[1] This work was aided by a grant from the Ella Sachs Plotz Foundation.

mean anatomical measurements of the group as recorded at death. The pregnant group were placed upon the special diet at the same time (166 days of age) and then 18 days later they were placed in breeding cages. All of the rats which were retained in the experiment cast their litter within the following 28 day period and hence had become pregnant within a time interval covering two oestrous cycles. Those which had had no young at this time were removed from the experiment. Eight days were thus allowed for recovery from the immediate effects of the pregnancy before examining the kidneys. The pups were removed from the mothers as soon as possible after birth and in no case were they allowed to nurse more

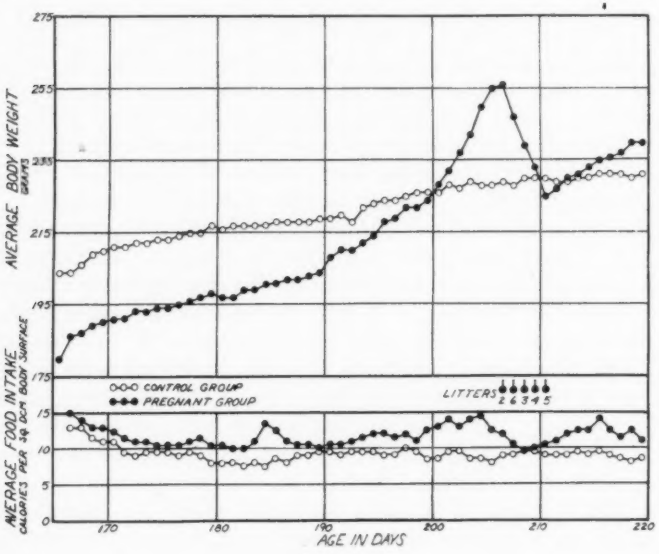

Fig. 1

than a very few hours. Each mother cast an average of 4.65 pups entailing a mean weight loss of 35 grams.

In figure 1 the daily food intake and body weight of the pregnant group have been compared with the same figures from the non-pregnant group. In table 1 are the mean kidney weights and other data obtained when the rats were killed. When these figures are compared with those of the control group in table 1 it will be seen that the kidneys are somewhat larger. This slight increase in the weight of the kidneys following pregnancy is however of no greater degree than the increase in the size of the organism as a whole. When the results are compared on the basis of milligrams of kidney weight per square decimeter of body surface the

probable difference between the means of the two groups is 2.0 mgm. while the actual difference is 1.0 mgm. We may then conclude that in young adult rats pregnancy increases the weight of the kidneys slightly but that the increase in renal weight parallels the increase in body surface showing that pregnancy has no specific effect on the kidney. Both the heart and the liver on the other hand are heavier following pregnancy. The actual increase in the mean heart weight: body surface ratio of the pregnant group in this experiment was 5.1 per cent in comparison with an expected difference of 1.1 per cent. The increase in the size of the liver is

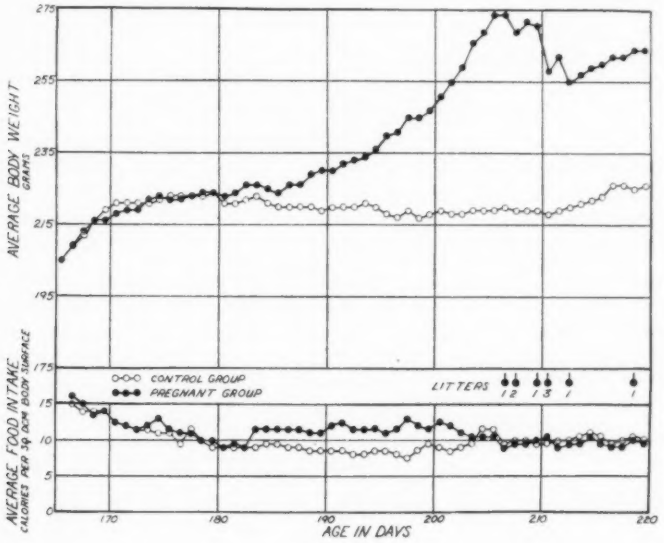

Fig. 2

even more striking in that there is an actual increase in the mean liver: body surface ratio of 19.5 per cent as against an expected difference of 2.7 per cent.

The effect of pregnancy in old rats—(Expt. 2). In the preceding experiment pregnancy in rats 180 days of age was found to have no effect on the weight of the kidney in relation to body surface. The actual renal weight did increase however so that its relation to body surface was maintained. Age has been shown (MacKay, MacKay and Addis, 1925) to be one of the factors which determines the degree of compensatory renal hypertrophy which follows the removal of one kidney and the question arose as to the possibility of older rats being less able to respond to the environmental changes of pregnancy. At 180 days of age female rats are at

TABLE 1

	NUMBER OF RATS IN EXPERIMENT	INITIAL BODY WEIGHT	FINAL BODY WEIGHT		BODY SURFACE	KIDNEY WEIGHT: AVERAGE	KIDNEY PER 100 SQ. CM. BODY SURFACE	HEART PER 100 SQ. CM. BODY SURFACE	LIVER PER 100 SQ. CM. BODY SURFACE
			Gross	Corrected					
Experiment 1. Control group (220 days old)									
		grams	*grams*	*grams*	*sq. cm.*	*mgm.*	*mgm.*	*mgm.*	*grams*
Mean	**19**	**204**	**231**	**226**	**421**	**606**	**144**	**156**	**1.74**
Standard deviation							9.8	8.8	0.17
Probable error							1.5	1.4	0.03
Coefficient of variability							6.8	5.6	9.8
Experiment 1. Pregnant group (220 days old)									
Mean	**20**	**180**	**240**	**231**	**426**	**617**	**145**	**164**	**2.08**
Standard deviation							10.6	7.1	0.26
Probable error							1.6	1.1	0.04
Coefficient of variability							7.3	4.3	12.5
Experiment 2. Control group (400 days old)									
Mean	**24**	**205**	**226**	**221**	**415**	**640**	**154**	**166**	**1.75**
Standard deviation							7.9	9.8	0.15
Probable error							1.1	1.4	0.02
Coefficient of variability							5.1	5.9	8.6
Experiment 2. Pregnant group (400 days old)									
Mean	**9**	**204**	**264**	**253**	**453**	**735**	**163**	**176**	**2.10**
Standard deviation							15.6	8.1	0.15
Probable error							3.5	1.9	0.04
Coefficient of variability							9.6	4.6	7.1
Experiment 3. Control (pregnant, non-lactating) group (220 days old)									
Mean	**19**	**186**	**231**	**227**	**422**	**614**	**146**	**158**	**1.84**
Standard deviation							11.1	8.4	0.14
Probable error							1.7	1.9	0.02
Coefficient of variability							7.6	5.3	7.6
Experiment 3. Lactating group (220 days old)									
Mean	**17**	**179**	**226**	**218**	**411**	**648**	**158**	**173**	**2.13**
Standard deviation							8.2	10.4	0.16
Probable error							1.3	1.7	0.03
Coefficient of variability							5.2	6.0	7.5

* In order to conserve journal space only mean figures have been given here. The author will be glad to furnish any worker who may have use for it with a copy of the original data.

an ideal breeding period and have great adaptability, so a second experiment was carried out on rats of approximately double this age when it was thought that the kidney might not keep up with the general increase in body size. This was not found, however, to be the case.

The mean daily food intake and body weight of the control group have been compared with those of the pregnant group in figure 2. Both groups were placed upon the special diet when 346 days of age. The pregnancy group were placed in breeding cages when 364 days old. Most of these rats proved sterile so that it was necessary to retain in the experiment any rat which cast a litter before the end of the period. When the rats of both groups were 400 days of age they were killed. The mean measurements are recorded in table 1. As before, the rats which had been pregnant had larger kidneys but the whole organism had also become larger. The actual difference between the mean kidney weight: body surface ratios of each group is 9.0 mgm. Since this is not greater than three times the probable difference (3×3.7) it is probably not significant. We may then conclude that pregnancy is also without any specific effect on the weight of the kidneys in old rats. The effect of this factor upon the weight of the heart and the liver was identical with that in the younger rats. An actual increase in the heart: body surface ratio of 6.0 per cent as compared to an expected difference of 1.4 per cent was found, and again the increase in the liver: body surface ratio is still the more significant, its actual increase being 20.0 per cent as against an expected difference of 2.5 per cent between the two groups of this experiment.

The effect of a preceding pregnancy—(Expt. 3—control group). This group was intended primarily to serve as a control for an experiment on the effect of lactation. It has however an interest of its own since a longer time was allowed after the termination of the pregnancy before the animals were killed. The special diet was commenced at the same time as in the first experiment, when the rats were 166 days of age. These differed in having been placed in breeding cages when 150 days of age and all had cast their litter before they were 180 days old. As in the preceding experiments the pups were not allowed to nurse but were removed from the cage immediately after birth. In figure 3 the mean daily food intake and body figures are compared with those of the control group for experiment 1 which also serves as the control group for this experiment. These rats also were killed when 220 days old. The mean anatomical measurements are presented in table 1. Neither the kidneys, heart nor liver, in relation to body surface, showed any significant change in weight. The actual difference between the mean kidney weight: body surface ratio of this experiment and that of the control group of experiment 1 is only 2.28 mgm. while the probable difference is 2.00 mgm. A pregnancy terminating 45 days beforehand is therefore without specific effect on the renal weight of

rats killed at 220 days of age, since the increase in the weight of the kidneys which is of the same magnitude and accompanies the increase in the weight of the organism as a whole (expt. 1) is still retained at this time. The heart and liver on the other hand, although absolutely and relatively increased in weight immediately after the end of pregnancy, here have decreased in weight, and now, like the renal tissue, bear the same relation to the body surface as they did previous to pregnancy. The actual differences between the heart: body surface and liver: body surface ratios are 2.0 mgm. and 0.10 gram respectively to be considered in relation to expected differences of 2.3 mgm. and 0.033 gram.

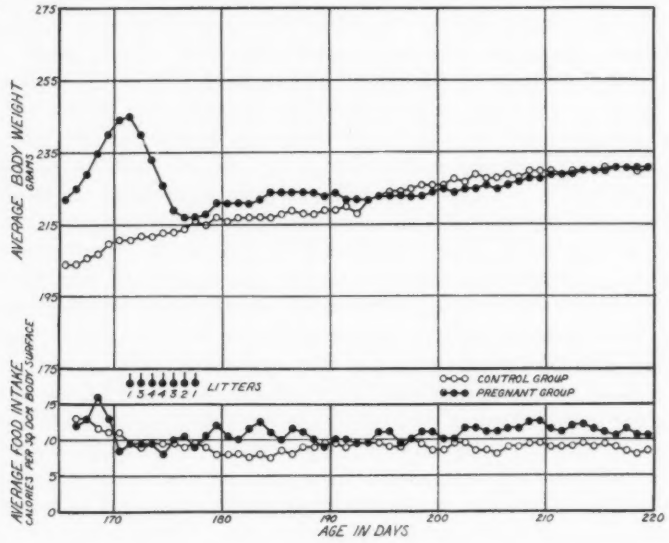

Fig. 3

The effect of lactation—(Expt. 3—lactating group). This group was identical with the one just preceding except that the young were left with the mothers after birth and allowed to nurse. This necessitated a slight change in the general procedure for each litter and its mother had to be removed to an individual cage at the birth of the young. Four pups were left in each litter and in case the number born to a mother was less than this some other young born on the same day were added to make the number up to four. When, as happened in a few instances, some of the young died later in the experiment they were replaced at once with others of the same age. Any rat which did not cast a litter before the fourteenth day of the experiment or was unable to nurse its young was discarded. A comparison of

this experiment and the preceding one as to the number of pups born to each mother and the body weight change is given in table 1. The anatomical data obtained when these lactating rats were killed at 220 days of age is included in table 1. It is evident that the lactation led to not only an absolute increase in the weight of the kidneys but also an increase in

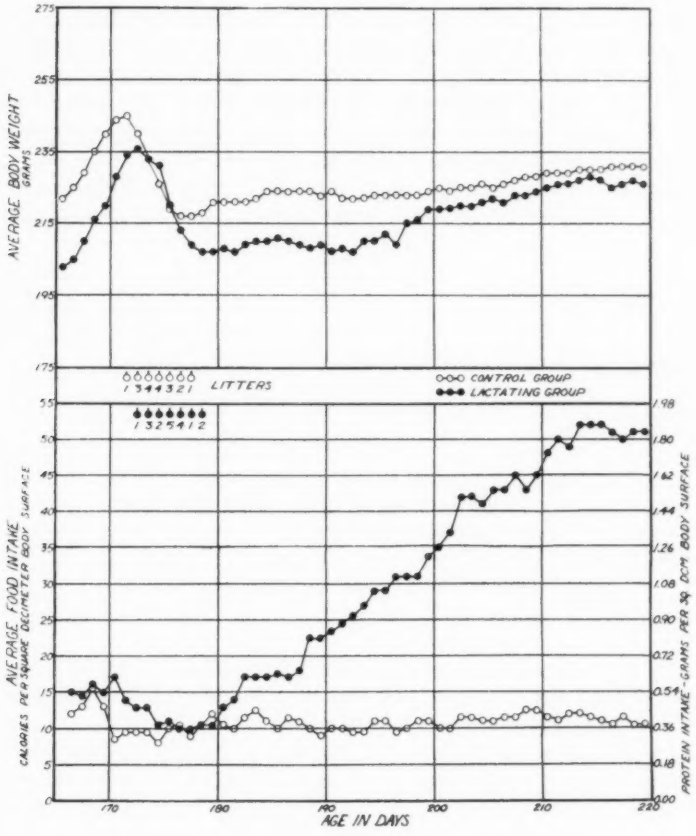

Fig. 4

kidney weight relative to body surface. In comparing this group with its control an average of 12 mgm. more of renal tissue per square decimeter of body surface is found. This is in contrast to a probable difference between the group means of 2.2 mgm. Lactation has then very definitely led to an increase in renal weight of 8.2 per cent.

An increase in the weight of the heart was found in rats which were

killed about 8 days after the normal termination of a period of pregnancy. When 45 days, during which there was no lactation, were allowed to elapse before examining the organs this increase in heart size had disappeared. But in this experiment where the mothers were allowed to nurse their young this increased heart weight not only remains but there is a possibly significant additional increase in the weight of the organ. In comparison with the increase in weight of 5.1 per cent observed immediately after a pregnancy there is now found an increase of 10.9 per cent while the expected difference is only 1.4 per cent. Unlike the heart there was no further increase in weight of the liver but the increase which was present immediately after pregnancy is retained. The actual increase in weight is 22.4 per cent with an expected difference of only 2.1 per cent.

The weights of the kidneys, heart and liver then were all increased by lactation. Whether these increases are transient and would disappear if

TABLE 2

	PREGNANCY WITHOUT LACTATION	PREGNANCY FOLLOWED BY LACTATION
Mean weight loss (grams) at birth of litter	35.5	38.3
Mean number of pups per mother	4.7	5.9
Number of pups allowed to nurse each mother	0	4
Mean food intake (grams) per square decimeter body surface per day	2.3	6.0
Mean calorie intake per square decimeter body surface per day	11.0	28.7
Mean protein intake (grams) per square decimeter body surface per day	0.4	1.0
Mean renal weight (milligrams) per square decimeter body surface at death	146.0	158.0

some time were allowed to elapse after the period of lactation, as the increased weight of the heart and liver were found to have done 45 days after the end of pregnancy, is undertermined.

In figure 4 the mean daily food intake and body weight of the lactation group and their controls have been charted. After the pups were born these lactating rats show a weight loss which was absent in the rats which were not allowed to nurse their young. This is recovered from, however, and by the end of the experiment the average weights are comparable with those of the control experiment. As might be expected, the food intake of the lactating group is higher than in any other experiment and it increases steadily throughout the period of observation. Beyond the fortieth day of the experiment the curve is not reliable as a measure of the mother's food intake for by this time most of the young were old enough to leave the nest and they undoubtedly made occasional visits to the food box.

We believe that the increased food consumption just described rather than the period of lactation itself was responsible for the increased renal weight in this group. The increase in food intake entailed an increase of 150 per cent in the protein consumption (table 2) and the ingestion of more than the usual amount of protein has been shown (Osborne and Mendel, 1924) to lead to an increase in the weight of the kidneys. This increased food, and hence protein intake, in the lactating group of course represents largely the food of the young, and the nitrogen of this part of the diet leaves the organism of the mother through the mammary glands and not the kidneys. However a high protein diet does not always cause renal enlargement by virtue of the increased nitrogen excretion required of the renal organs for an equivalent excess of nitrogen, above a normal protein intake, in the form of urea causes no such increase in the size of the kidneys (MacKay, MacKay and Addis, 1927). This in turn suggests that the increase in kidney weight which accompanies the ingestion of a high protein diet is the result of the circulation of a higher concentration of protein split products in the organism. Data on this point are not available but such a condition may easily exist in lactating animals.

SUMMARY

The kidney weights of two groups of albino rats, killed when 220 days of age, were not affected by pregnancies which had terminated for one group at approximately 8 days previously and for the other at about 45 days before. The kidneys of the pregnant animals were slightly increased in weight but this enlargement was of the same degree as the increase in the size of the organism as a whole. On the other hand, the heart increased in weight 5.1 per cent and the liver 19.5 per cent, both in relation to the body surface, in the group examined 8 days after pregnancy. When 45 days were allowed to elapse this increase in weight had disappeared.

Age has no effect upon the capacity of the kidney to keep pace with the enlargement of the whole organism which results from pregnancy, for a pregnancy terminating just before death in rats which were killed when 400 days old had produced no effect upon the weight of the kidneys in relation to body size. Almost identical with the changes found in the younger rats were the increases in the heart weight of 6.0 per cent and the liver weight of 20.0 per cent over that of the body as a whole.

A 45 day period of lactation ending just before death in albino rats killed when 220 days of age caused an increase in the weight of their kidneys of 8.2 per cent more than the increase in the size of the organism as a whole. The high food intake of the lactating group suggested that this renal hypertrophy was the result of a high protein intake rather than due to the lactation itself. The increase in liver weight in relation to body surface of 19.5 per cent found just after pregnancy, but which had disappeared 45

days later, was retained (22.4 per cent) when the animals were allowed to lactate. The increase in the weight of the heart in proportion to body size of 5.1 per cent, due solely to pregnancy, was not only retained but further increased to 10.9 per cent by the period of lactation.

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THE INORGANIC PHOSPHORUS OF BLOOD AND MUSCLE

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Since Embden's proposition that lactacidogen is a precursor of lactic acid which likewise yields phosphoric acid in decomposing (1), there have been many attempts to show that the acid soluble inorganic phosphorus of muscle increases during contraction. A small increase could be shown in contracting frog muscle (2), but the condition reported in mammalian muscle is not so clear. After exhausting muscular work (3), (4), (5) it appears that the acid soluble inorganic phosphorus in the muscle is increased, but in some cases, apparently where the degree of muscular fatigue is less, no increase or even a decrease may occur.

The situation was altered by the announcement of Eggleton and Eggleton (6) that most of the inorganic phosphorus of muscle originates from decomposition of a labile organic compound. The trichloracetic acid extract from a normal muscle contains inorganic phosphorus and a greater amount of labile organic phosphorus which quickly decomposes at room temperature with release of its phosphorus in acid soluble inorganic form. Fiske and Subbarow (7), (8) showed that the labile phosphorus occurred in a compound of phosphoric acid and creatine, which they designated phosphocreatine. This labile compound is expended during contraction of isolated muscles with resulting increase in the concentration within the muscle of acid soluble inorganic phosphorus.

Because of the likelihood that contraction in normal working mammalian muscles would increase the true acid soluble inorganic phosphorus concentration, Eggleton and Eggleton (9) suggested that the increase of inorganic phosphorus in the blood after muscular exertion originated in the muscles.

The subject of whether phosphorus of blood and urine is in equilibrium with muscle phosphorus is also of special interest in relation to carbohydrate metabolism. It has been shown by Sokhey and Allan (10) that after insulin, a transient decrease followed by an increase in the urinary excretion of phosphorus occurs but that this fails to appear in the depancreatized animal (11). After muscular exercise the blood inorganic phosphorus is increased (12), while under the influence of insulin a decrease is evident (13), (14), (15). The prevailing attitude has been to ascribe the inorganic phosphorus increases in blood and urine to muscular activity,

but no results could show the expenditure of any considerable amount of lactacidogen in mammalian muscles short of complete exhaustion. Upon considering the phosphocreatine of muscle, however, two important points appear: *1.* The disparity between the concentration of inorganic phosphorus in resting muscles and in blood is less than was formerly thought. In dog blood, the inorganic phosphorus amounts to about 0.04 to 0.06 mgm. per milliliter, in muscle, excluding phosphocreatine, about 0.15 to 0.25 mgm. per gram. *2.* Without utterly exhausting the muscle it is possible to double its true inorganic phosphorus concentration. As the increased inorganic phosphorus concentration is apparently attainable under physiological conditions, we are justified in examining the venous blood from a working muscle to see if its phosphorus concentration is greater than that of arterial blood.

With this in view the following described experiments were arranged to change the muscle inorganic phosphorus concentration and determine if the changes were reflected in the blood. As controls for estimating the alteration of the muscles, lactic acid and moisture determinations were also made.

ANALYTICAL METHODS. *Phosphocreatine and inorganic phosphorus.* Samples from muscle ground in liquid air were extracted in ice-cold 10 per cent trichloracetic acid for one hour. In the filtrate phosphorus was determined by the Fiske and Subbarrow (16) colorimetric method against a standard simultaneously prepared and containing an equivalent quantity of cold trichloracetic acid. As Fiske and Subbarrow (7) and Eggleton and Eggleton (6) described, the color develops more slowly in a fresh ice-cold muscle extract than in the standard. Extrapolation of the curve for colorimeter reading against time to zero time shows the initial amount of inorganic phosphorus. The final attainment of constant readings represents the original inorganic phosphorus plus that derived from decomposition of phosphocreatine, and so indicates the quantity of the latter by difference. Although we felt highly skeptical as to the accuracy of the values for inorganic phosphorus at zero time, on account of the steepness of the extrapolated curve, many duplicate determinations showed good agreement. While the agreement of duplicate end points was to within 4 per cent, that of early points might vary twice as much, so that the extrapolated zero time values often differed by 10 per cent in duplicate.

Lactic acid was determined in the Schenck filtrate from the frozen muscles by the method of Friedman, Cotonio and Shaffer (17).

Dry substance was determined by heating to constant weight at 104°–5°C.

Phosphorus determinations on whole blood were made according to Fiske and Subbarrow's (16) method, using 2 ml. of freshly drawn blood pipetted into 10 per cent trichloracetic acid and filtered immediately.

The first experiments, recorded in table 1, show that one hour after death

in situ the phosphocreatine was by no means decomposed. The initial lactic acid values indicate the condition of the muscles, i.e., that the rabbit muscle was already in poor condition, while the cat muscle remained in excellent shape. An inverse relation between amount of lactic acid present and phosphocreatine generally appeared, but as most of the experiments dealt with muscles with intact circulation, there is no evidence that the relation was other than incidental.

The results of stimulating muscles to exhaustion are shown in table 2. The phosphocreatine of the resting muscle was about 60 per cent of the total inorganic phosphorus, less than was found in the best preparations, and the lactic acid content was about three times that of the best. Such an accumulation of lactic acid and gain in water as occurred in the worked muscle are extreme for muscles, particularly with intact circulation. The

TABLE 1

Post mortem changes in muscle

	LACTIC ACID PER GRAM	PHOSPHOCREATINE—PHOSPHORUS PER GRAM	ACID SOLUBLE INORGANIC PHOSPHORUS—PHOSPHORUS PER GRAM
	mgm.	*mgm.*	*mgm.*
Cat 3a. Semi-membranosus:			
Immediate	0.28	0.84	0.08
After 1 hour	1.92	0.60	0.35
Rabbit 2.* Gastrocnemius:			
Immediate	1.53	0.35	0.56
After 1½ hours	2.35	0.13	0.80

* Died under amytal anesthesia.

animal died at the end of the experiment, while the muscle still responded perceptibly to direct electrical stimulation, so that a general moribund condition contributed to the exhaustion. We see that the inorganic phosphorus was more than doubled, so that conditions are attainable which will favor the examination of the effect of increased phosphorus concentration in muscle upon that in the blood.

Not only was the inorganic phosphorus increased at the expense of phosphocreatine, but also from an additional source, as was noted by Eggleton and Eggleton in fatigued frogs' muscles. This increase in the inorganic phosphorus beyond that derived from phosphocreatine is ascribable to decomposition of lactacidogen, which evidently occurs only when muscle is really exhausted (5).

In the last experiment of table 2, a number of causes might have con-

tributed to exhaustion, among which the most conspicuous is anoxemia from failing circulation. Restricting the circulation of contracting muscles for a brief time has been shown to accentuate the characteristic fatigue changes of increased water content of muscle without affecting the lactacidogen (5), and Eggleton and Eggleton found an anaerobic disappearance of phosphocreatine in excised frog muscle, with subsequent aerobic recovery. Obviously the effect can not properly be ascribed in

TABLE 2

The effects of exhaustion upon the muscle phosphorus

EXPERIMENT NUMBER		LACTIC ACID PER GRAM	PHOSPHOCREATINE—PHOSPHORUS PER GRAM	ACID SOLUBLE INORGANIC PHOSPHORUS PER GRAM	DRY SUBSTANCE
		mgm.	*mgm.*	*mgm.*	*per cent*
14	Resting	0.82	0.56	0.40	25.2
	Worked	3.55	0.15	0.99	22.5

Gracilis muscles of decerebrate cat contracting isotonically from 60 short tetanizing stimuli per minute through nerve for 24 minutes.

TABLE 3

The effect of restricted circulation upon muscle phosphorus

EXPERIMENT NUMBER		LACTIC ACID PER GRAM	PHOSPHOCREATINE—PHOSPHORUS PER GRAM	ACID SOLUBLE INORGANIC PHOSPHORUS PER GRAM	DRY SUBSTANCE
		mgm.	*mgm.*	*mgm.*	*per cent*
5	Gracilis—open	0.51	0.75	0.10	24.21
	Gracilis—restricted 30 minutes	1.85	0.48	0.36	22.12
	Semitend.—open	0.94	0.62	0.32	24.52
	Semitend.—restricted 40 minutes	1.21	0.37	0.40	25.35
3	Gracilis—open	0.31	0.93	0.10	23.58
	Gracilis—restricted 20 minutes	0.47	0.90	0.10	23.31

Decapitate cats; circulation restricted by pulling on a loose ligature.

our experiments with contracting muscles, to failing circulation alone. Consequently in the experiments recorded in table 3, we determined to restrict the circulation of resting muscles artificially. The circulation to the resting muscles was reduced by drawing upon a loose ligature under the principal vessels. Restriction of the circulation increased the lactic acid content of the muscle and diminish the phosphocreatine correspondingly. So the gracilis of experiment 3 had only a small lactic acid and water increase, and no perceptible phosphocreatine loss. The gracilis

of experiment 5, restricted for a longer time, had conspicuously gained in lactic acid and water while losing phosphocreatine. The semitendinosus muscle during restriction had made only a slight gain in lactic acid, while losing considerable phosphocreatine and apparently losing water. Whereas, the gracilis muscle suffered increase of inorganic phosphorus, without any net loss from the muscle, the semitendinous had a smaller total of inorganic plus phosphocreatine phosphorus. Either some had escaped

TABLE 4

Phosphorus in blood and muscle after exhaustion. Cat gastrocnemii in situ with isolated circulation, and vein cut

CAT NUMBER		LACTIC ACID PER GRAM	PHOSPHOCREATINE PHOSPHORUS PER GRAM	ACID SOLUBLE INORGANIC PHOSPHORUS PER GRAM	DRY SUBSTANCE	BLOOD INORGANIC PHOSPHORUS		
							Before stimulation	After stimulation
		mgm.	*mgm.*	*mgm.*	*per cent*		*mgm. P. per ml.*	*mgm. P. per ml.*
7*	Resting	0.91	0.63	0.20	25.6	Arterial	0.037	0.042
						Venous, rest‡		0.055
	Stimulated 40 minutes at 60 per minute	0.93	0.0	1.21	25.0	Venous, work‡	Flow good	0.052
1*	Resting		0.41	0.50	23.5	Arterial	0.037	0.034
						Venous, rest‡		0.030
	Stimulated 30 minutes at 15–20 per minute (slow circulation)		0.02	0.76	20.6	Venous, work‡		0.035
6†	Resting	0.75	0.51	0.24	25.9	Arterial	0.049	0.084
						Venous, rest‡		
	Stimulated 40 minutes at 60 per minute	3.82	0.08	0.96	24.5	Venous, work‡		0.069

* Decapitate.

† Amytal anesthesia.

‡ Sample from whole outflow.

into the blood or had been combined in another form, for example lactacidogen.

Considering the disparity between blood and muscle inorganic phosphorus concentration, a more sensitive criterion for loss of inorganic phosphorus is the comparison of arterial and venous blood, in order to see if the muscle phosphorus diffuses out. Even with rapid circulation, if diffusion is free, the effect of exposure to inorganic phosphorus concentration in the muscle ten times as great as in the blood, should easily be detected.

To examine this proposition the gastrocnemii of decapitated cats were dissected free from surrounding structures except for the insertion at the head, denervated, and contributing venous branches tied off so that only blood from the gastrocnemii returned through the popliteal veins. At the start of the experimental period, the vein was cut and the blood collected from both muscles while one was stimulated through the nerve. Arterial blood samples were drawn from the carotid artery at the start and end, while samples from the oxalated venous outflow showed its average composition. The analytical results are reported in table 4.

The results are from animals which are on the whole in poor condition by the end of the experiment, on account of exposure and handling during the rather difficult preparation. The collection of outflowing blood was also complicated by clotting, so that the muscle circulation was poor. Still the resting muscles do not have a very high lactic acid content and their phosphocreatine is not much less than the best values observed. The worked muscles also responded over considerable periods with vigorous contractions; so that while showing the strain of manipulation, they are not moribund.

The desired condition is attained in the experiments in that the muscle inorganic phosphorus is shown to be much greater in the worked than in the resting muscles. Comparison of the arterial and venous blood does not show a significant increase in inorganic phosphorus. In experiment 7 both resting and worked venous bloods were above the arterial value, but there was no difference assignable to the effect of muscle contraction. In experiment 1 the venous blood from the resting muscle had less phosphorus than the worked, but both venous values were less than the average for arterial blood. Again, in experiment 6 the phosphorus of the worked muscle's venous blood was about the average of the rapidly increasing arterial blood.

The experiments do not prove than no increase of blood inorganic phosphorus occurred during passage through a working muscle. They do show that there is no significant change in the blood in respect to inorganic phosphorus commensurate with the change in inorganic muscle phosphorus concentration.

The worked muscles were severly fatigued, as judged by physical signs and by the accumulation of lactic acid and water, and by the evident breakdown of lactacidogen in nos. 7 and 6. That the venous blood was significantly affected appears from the moisture determinations upon the blood from nos. 7 and 6 (table 5). Water had been withdrawn from the blood during its passage through working muscles. Himwich and Castle (18) indirectly concluded a loss of water from blood passing through a resting muscle by comparing the oxygen dissociation curves.

A series of similar experiments (table 6) with dogs yielded results com-

parable to those with cats. They are also open to the same objections, inasmuch as the operative procedure alone severely affected the animals,

TABLE 5

Changes in water content of blood during passage through a muscle

		DRY SUBSTANCE	
		Resting	Worked
		per cent	*per cent*
Cat 7	Arterial blood	19.9	19.4
	Venous blood	19.9	21.1
Cat 6	Arterial blood	19.60	18.0
	Venous blood		21.0

TABLE 6

Phosphorus in blood and muscle. Dog gastrocnemius in situ with isolated circulation

DOG NUMBER		LACTIC ACID PER GRAM	PHOSPHOCREATINE—PHOSPHORUS PER GRAM	ACID SOLUBLE INORGANIC PHOSPHORUS PER GRAM	SUM OF COLUMNS 2 AND 3	BLOOD INORGANIC PHOSPHORUS			
							Resting		After work
							Before	After	
		mgm.	*mgm.*	*mgm.*			*mgm. per ml.*	*mgm. per ml.*	*mgm. per ml.*
8*	Resting		0.33	0.18	51	Arterial		00.55	1.05‡
						Venous, rest	0.053	0.053	
	Worked 10 minutes at 60 per minute direct stimulus		0.09	0.39	48	Venous, work		0.060	0.085
9*	Resting	0.28	0.51	0.16	67	Arterial	0.040	0.032	0.037
						Venous, rest	0.042	0.031	
	Worked 36 minutes at 60 per minute	0.29	0.28	0.21	49	Venous, work	0.042	0.032	0.034
4†	Resting		0.24	0.47	71	Arterial	0.060		0.063
						Venous, rest			
	Worked 30 minutes at 30 per minute		0.16	0.44	60	Venous, work			0.056

* Amytal.
† Decapitate.
‡ Resting.

although in the one experiment where muscle lactic acid was determined there had been no accumulation of lactic acid; unfortunately, no other

control determinations were made. The muscles were not completely fatigued, and showed in each case a decrease in the sum of phosphocreatine and inorganic phosphorus during work, a change previously (p. 225) suggested as frequent after moderate work. We must, therefore, assume that either phosphorus has been lost to the circulation, or that it has been fixed in another organic compound than phosphocreatine in the muscle.

Considering the one of these possibilities upon which we have direct evidence, it appears in experiments 9 and 4 that the venous blood has certainly not gained inorganic phosphorus. In the venous blood in experiment 8 we showed a rapidly increasing inorganic phosphorus content, but the final arterial blood sample also contained so much that the effect is best ascribed to other factors than the fatigue of the single muscle. The venous blood only showed points on a rapidly rising curve for the phosphorus concentration of all of the circulating blood. There is no evidence of an outflow of inorganic phosphorus from the muscle into the blood.

To determine if the disappearance of inorganic phosphorus from these moderately worked muscles resulted in an increase in lactacidogen, a number of determinations have been made of the inorganic phosphorus in muscle after incubation at 40 and 42°C. in 2 per cent sodium bicarbonate solution for two hours. In experiment 9 the resting muscles after incubation contained 1.00 mgm. of P per gram, the worked 1.02,—no difference. The lactacidogen had, therefore, increased during moderate work by reducing the sum of inorganic phosphorus and phosphocreatine. In the light of other experiments, however, we are not confident that the inorganic phosphorus determination after incubation is dependable as an indication of total lactacidogen decomposition. As far as the results appear reliable, moderate treatment of the muscle did not affect lactacidogen by altering the amount of phophorus present after incubation. We can, therefore, infer that lactacidogen is increased to account for the loss of phosphorus from the moderately worked dog muscle.

In the last series of experiments dogs under amytal were used with minimal exposure of the muscles. The Achilles tendon was exposed, severed and attached to a loaded isotonic lever. Where the tibialis anticus muscles were used, their tendons could not be severed on account of hemorrhage from the accompanying vessels, so they worked only against the antagonistic muscles. Stimulation was applied through Sherrington electrodes to the peripheral end of the cut sciatic nerves. Blood samples were drawn from the carotid artery and from the femoral vein by a needle inserted through the saphenous branch. The adjacent branches from the gracilis and semitendinosus muscles were tied off, so that blood drawn came principally from the lower leg.

The muscles were worked with the object of inducing some fatigue, when the first muscle was removed; the other was then allowed a recovery period.

The muscles are arranged in order in table 7 according to degree of fatigue as estimated by diminished height of contraction. In experiment 11 the contraction height was diminished by about 40 per cent; the others suffered less fatigue, until no. 16 was not perceptibly fatigued after 2 hours and 40 minutes' occasionally interrupted tetanus with about a 5 pound load. The phosphocreatine content of the last pair of muscles is particularly great, the lactic acid content is low, and there is no moisture difference.

TABLE 7

Effect of work and recovery upon muscle. Dog muscles in situ—partially isolated—stimulated through nerve

EXPERIMENT NUMBER		LACTIC ACID PER GRAM	PHOSPHOCREATINE PHOSPHORUS—PER GRAM	ACID SOLUBLE INORGANIC PHOSPHORUS PER GRAM	DRY SUBSTANCE
		mgm.	*mgm.*	*mgm.*	*per cent*
11*	Worked 1 hour 23 minutes at 60 per minute	0.84	0.33	0.22	25.7
	Recovery 1 hour 21 minutes	0.76	0.34	0.31	23.9
15*	Worked 43 minutes at 70 per minute	0.37	0.46	0.20	23.9
	Recovery 1 hour 52 minutes	0.23	0.42	0.27	24.8
12†	Worked 20 minutes at 55 per minute	0.56	0.44	0.19	24.8
	Recovery 1 hour 12 minutes	0.28	0.49	0.20	26.4
13†	Worked 1 hour 41 minutes at 120 per minute	0.68	0.54	0.16	25.0
	Recovery 1 hour 19 minutes	0.59	0.50	0.20	26.0
16*	Worked 2 hours 40 minutes tetanus	0.27	0.51	0.12	22.8
	Recovery 1 hour 26 minutes	0.26	0.55	0.20	22.7

* Gastrocnemius.

† Tibialis anticus.

We would, therefore, consider that these muscles were not in any way fatigued. The first muscles have less phosphocreatine, but in none is there a large amount of lactic acid present.

Such muscles did not have far to recover, and did not appreciably regain phosphocreatine during the recovery period. The small decrease in lactic acid was hardly significant because of the small amount available for analysis, but it was consistently less after recovery. Except for no. 11 and the least fatigued, no. 16, the muscles lost water during recovery,

thereby reversing the change characteristic during contraction. There is the most striking evidence for recovery change.

Although no increase in phosphocreatine occurred during recovery, the concentration of inorganic phosphorus was uniformly greater after recovery.

TABLE 8

Inorganic phosphorus of blood (arterial and venous) during work and subsequent rest

Numbers indicate correspondence to muscle experiments in table 7, expressed in milligrams phosphorus per liter of blood.

EXPERIMENT NUMBER	BLOOD	BEFORE WORK	DURING WORK	AFTER WORK (RECOVERY)
11	Arterial	53	53	62
	Venous*	57	64	
	Venous*			71
15	Arterial	41	41 39	41 47 47
	Left venous	36	40 40	
	Right venous	42	46 42	40 40 47
12	Arterial	42	39	37 37 37
	Left venous	36		38 39 34
	Right venous	43	41	
13	Arterial	49	47 49	51 49 53
	Left venous	47	46 50	
	Right venous	45	47 49	49 32 54

* Circulation impeded by clots.

TABLE 9

Inorganic phosphorus of blood (arterial and venous) during work and subsequent rest, and of blood after short venous occlusion

Experiment 16. Phosphorus in milligrams per liter blood

BLOOD	BEFORE WORK	DURING WORK	DURING RECOVERY
Arterial	64 74	68 68 70	65 71 68
Left venous	67 75	68 69	69 71
Left venous occluded	73	65	71 64 66
Right venous	64 74	70 65	
Right venous occluded	63	68 66	

Corresponds to muscles of experiment 16.

In considering the effect of this change in terms of lactacidogen, there would be indicated a loss of lactacidogen, during recovery and a gain during contraction. It was already suggested in discussing table 6 that moderately worked muscles had more lactacidogen than their resting controls. This

view is quite consistent with the supposed position of lactacidogen in the carbohydrate metabolism of muscle.

The record of inorganic phosphorus determinations on the blood samples in these experiments (tables 8 and 9) did not show any significant increase during prolonged muscular work and recovery, although the inorganic phosphorus concentration of the muscles became greater during recovery. There were changes in arterial blood exceeding the range of analytical error, so that the blood phosphorus was not constant, but the venous blood did not gain phosphorus in passing through either working or recovering muscles.

Frequently it had been observed (expt. 6, table 4; expt. 8, table 1) that the inorganic phosphorus concentration of the arterial blood steadily rose and became particularly great at death. While there was no evidence of the source of the phosphorus, it seemed that anoxemia might be the cause of its increase. Accordingly, after experiment 11 the trachea was clamped for about five minutes, until the heart failed, and a last blood sample drawn from the heart. The inorganic phosphorus concentration was 0.077, compared with 0.062 mgm. per milliliter in the sample five minutes before asphyxia. The phosphorus content of this dog's blood was already rising before the asphyxia, and asphyxia leading to death involves other secondary effects.

To determine if brief stasis of blood in muscle caused an outflow of phosphorus, during the course of experiment 16 the vein was completely occluded for five minute periods several times. The completely stagnant blood in muscle during work and recovery differed only once out of seven times from the normal venous outflow so that stagnation was apparently without significant influence. During the period of blood stagnation, relaxation of the contracting muscle was just perceptibly slower, and the stagnant blood drawn was very dark, so that anoxemia must have been severe in the muscle.

It would have been desirable to fatigue a muscle completely while its circulation and general condition remained good, but we were not able to induce fatigue in well circulated muscles of animals in good condition. It was likewise only in muscles either themselves suffering from manipulation or belonging to weakening animals that the phosphocreatine could be expended. While many of these animals still maintained a steady inorganic phosphorus concentration of their arterial blood, it increased rapidly in moribund animals. In such a state it is hard to establish as a fact that no increase occurs during passage through the muscles. We can say with certainty, however, that in all cases not approaching complete exhaustion, even under the stress of performing considerable work, that the muscles did not appreciably influence the inorganic phosphorus concentration of blood.

If the value obtained by extrapolation represents the true concentration of inorganic phosphorus in muscle, it is apparently never half as great in blood, and the ratio during fatigue may fall to one-tenth. With this view it is necessary to think that no close equality exists between blood and muscle inorganic phosphorus. There is possibly a sudden combination in organic form upon entering the blood, for the organic phosphorus of blood is normally about eight times as much as the inorganic. Still we have no indication from the muscle that it loses appreciable amounts of phosphorus under any conditions, and it appears that the inorganic phosphorus of muscle passes normally through an exclusively intramuscular cycle.

Some extreme conditions may bring about the interchange of phosphorus between blood and muscle, but we should be justified in seeking the changes in blood inorganic phosphorus which undoubtedly do occur after exercise (12) and during sleep (19) in other organs, or even in changes within the blood itself.

In considering this problem we have frankly avoided discussion of the true inorganic phosphorus concentration in muscle. Serum is normally saturated with phosphates (20) and it is possible that the greater concentration found in muscle exceeds the amount soluble, so that we are dealing with unstable organic compounds in the fraction termed inorganic. On that possibility we have no satisfactory evidence, but it does not hinder using the results of phosphorus determinations as indicators of the changes in which they occur.

SUMMARY

As lactic acid is produced in fatigued muscle, phosphocreatine decomposes, with consequent increase of inorganic phosphorus.

Under extreme fatigue conditions the increased inorganic phosphorus cannot have originated entirely in phosphocreatine. Probably the extra phosphorus originates in lactacidogen. Restricted circulation likewise causes phosphocreatine decomposition.

When fatigue is not reached, either because of moderate contractions or adequate circulation, the increased inorganic phosphorus is less than the decomposed phosphocreatine would indicate. Apparently some phosphorus is resynthesized in organic form, presumably lactacidogen. The reverse effect—gain in inorganic phosphorus—occurs during recovery, indicating a loss of lactacidogen.

In no case is there a gain in the inorganic phosphorus of venous blood from muscle, although the muscle may have had more than twice the normal concentration in the blood.

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EFFECT OF FATIGUE UPON THE CIRCULATION AND FIBERS OF SKELETAL MUSCLE

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Krogh has made a careful study of skeletal muscle capillaries following various conditions of activity. Much of the work was done on the frog. Direct observations in the living urethanized guinea pig were made with reflected light. He found that the capillaries were too narrow to be readily seen but that the blood moving through them indicated their presence. Stimulation of the muscle caused a considerable increase in the number of open capillaries.

We have been able to observe the capillaries in the sartorius muscle of the cat under the 4 mm. objective with transmitted light. This has enabled us to see the capillary wall. When one uses the presence of corpuscles in the capillaries to determine whether they are open, he really is only able to tell whether corpuscles are present. The capillaries may be dilated but the precapillary vessel constricted sufficiently to prevent passage of corpuscles, the so-called plasma skimming. Until we were able to see the capillary wall we frequently thought that the capillaries were closed when really, as later evidence has shown (2) they were not constricted. The arteriole or precapillary vessel, however, was constricted.

With this improved technique for observation we have undertaken the study of muscle during and following different conditions of activity. The direct effects of activity on the muscle itself as well as indirect effects of fatigue products have been followed.

METHODS. Fifteen young cats under the influence of ether or else decerebrated were employed in this study. All observations were upon the sartorius muscle with the 4 mm. objective. Transmitted light was furnished by a method previously described (Hartman, Evans and Walker).

For direct stimulation the femoral nerve was exposed just inside of the abdominal cavity. In some instances the nerve was cut and in others it was left intact. A large inductorium with two dry cells furnished the current which was interrupted by a vibrator in the primary circuit.

For the effect of substances carried by the blood during fatigue the peripheral end of the cut brachial plexus was stimulated. Lactic acid, hydrochloric acid and carbon dioxide were administered in a few experi-

ments. The hydrochloric acid was injected in order to compare another acid with lactic acid.

OBSERVATIONS. *Direct stimulation.* The sartorius stimulated through the femoral nerve was observed in 4 cats while anesthetized with ether. The following illustrates the results: *Cutting of the femoral nerve* caused the field to become much brighter: All vessels were dilated and there was continuous twitching of the muscle fiber. These effects, except the vascular dilatation, passed away shortly.

Stimulation with the *coil at 300 mm.* for 20 seconds produced a very bright field, dilatation of the capillaries, very rapid circulation and continuous transverse twitching.

Stimulation with the *coil at 250 mm.* for 7 minutes. The results were similar to the above except that some of the arteries and veins showed beading. Twitching of the muscle fibers and dilatation of the capillaries continued throughout the period of stimulation; striations were very clear.

Stimulation with the *coil at 200 mm.* for 25 minutes. Observations under the microscope were impossible while the stimulation was in progress because of the marked vibration or contraction of the muscle fibers.

After stimulation had ceased the field was observed to be brighter, all capillaries were open and dilated; striations were pronounced; from time to time there was epinephrin-like twitching which was accompanied by greater brightness. This condition lasted for more than eight minutes.

Stimulation of the brachial plexus. The sartorius of three cats anesthetized with ether was observed after stimulation of the peripheral end of the cut brachial plexus. The following was a typical result: The inductorium was set at 150 mm. and stimulation carried on for ten minutes. At 90 seconds after beginning the stimulation the circulation increased in the sartorius and the capillaries dilated. In 210 seconds new capillaries opened and the circulation increased tremendously. Fibrillar twitching began. The field grew brighter until by 400 seconds it was extremely bright.

In one cat the vascular dilatation was measured. Stimulation for 2.5 minutes with the coil at 150 mm. increased the capillaries from 3.7μ to 4.92μ and the arterioles from 6.15μ to 8.2μ. A vein alternately dilated and constricted from 70μ to 88μ. In 4.5 minutes one arteriole had decreased from 26.4μ to 15.8μ. Sometimes the dilatation was attended by a slower circulation.

Four decerebrate cats were studied. The results were similar.

It was soon realized that the reactions were identical with those produced by epinephrin. Two examples will illustrate. Stimulation of the brachial plexus for three minutes with the coil at 250 mm. in a 750 gram cat caused an increase in the circulation at 90 seconds. In 100 seconds

the twitching was undoubtedly of the epinephrin type. The field became brighter (110 seconds) and in 160 seconds the dilatation of the smaller vessels and the twitching of the muscle fibers had become very pronounced.

Twenty seconds after the stimulus ceased the effects began to wear off, in the form of decreasing brightness and lessened twitching although there was further dilatation as late as 225 seconds.

In another decerebrate cat (1980 grams) in which the brachial plexus was stimulated for twenty minutes with the coil at 50 mm. the following results were obtained:

The earlier effects were the same as those just described; dilatation of capillaries and a very bright field. By eleven minutes, however, the circulation became slow and finally stopped in all capillaries. Then in 12.25 minutes the circulation began again; the field became brighter; the muscle striations were extremely distinct; the capillaries dilated further and there was epinephrin-like twitching. There was also twitching unlike epinephrin twitching. Six and one-half minutes after stimulation stopped the capillaries were still dilated but the field was not so bright.

In order to rule out secretion from the adrenals, both glands were removed in three cats (ether anesthesia throughout) before the experiment was started and both sympathetic chains were cut below the diaphragm for stimulation later.

In two cats the field became cloudy instead of brighter due to brachial plexus stimulation. The capillaries dilated and there was a peculiar twitching unlike that produced by epinephrin. The circulation became slower or even stopped. Some observations in one of these follow:

Stimulation for 70 seconds at 300 mm.—Dilatation of capillaries and transverse twitching of muscle fibers

Stimulation for 3.5 minutes at 150 mm.—4 minutes after stimulation stopped, circulation slow and jerky, capillaries dilated
5 minutes 25 seconds—peculiar twitching in the muscle

Stimulation for 10 minutes at 100 mm.—Capillaries dilated. Violent twitching
4 minutes 45 seconds (after start) field cloudier
5 minutes, field much darker
6 minutes 15 seconds, circulation stopped
10 minutes 25 seconds, very slow circulation

The third cat, with the adrenals removed, gave the same results as those obtained in cats with intact adrenals, i.e., dilatation of the capillaries, clearing of the field and epinephrin-like twitching.

Lactic acid. Lactic acid was injected intravenously into four etherized cats. The following experiment is typical:

Cat, ♀, 1300 grams

Lactic acid	*Response in sartorius*
0.5 cc. 1:5,000	Increase in the circulation, dilatation of the small vessels (capillaries increased from 4.1μ to 6.2μ). Caliber of all capillaries not uniform. Field becomes slightly cloudy
1.0 cc. 1:5,000	As above but new capillaries open and the circulation is stopped for a short time
0.5 cc. 1:100	As above but some continuous transverse twitching; respirations increased, field slightly brighter but very hazy

Hydrochloric acid. In order to test further the effect of increased H-ion, hydrochloric acid was injected into three cats, in doses ranging from 1 cc. 1:1,000 to 1 cc., 1:100. Capillaries and other small vessels were dilated and the circulation was increased. In two animals there was violent longitudinal twitching of the muscle fibers. One cubic centimeter 1:100 caused dilatation of a venule from 12.3μ to 20.5μ and a vein from 17.6μ to 21.1μ. The capillary dilatation from this dose lasted for six minutes.

Carbon dioxide. The carbon dioxide in the blood was increased in an etherized cat by increasing the dead space for four minutes, a two-foot rubber tubing being attached to the tracheal cannula for the time. In two minutes, the field became cloudy and the capillaries, venules and arterioles were dilated.

In a second cat a rubber bag containing CO_2 was loosely connected with the tracheal cannula for 15 seconds. There was labored breathing for several seconds. In 38 seconds the capillaries were dilated and the circulation stopped. In 80 seconds there was reversal in the flow of blood in some capillaries. The field became hazier and the fibers yellowish. In 105 seconds the circulaton appeared normal. In 120 seconds the circulation was much increased. A small vein was constricted from 34μ to 26.5μ and a vein dilated from 70.4μ to 79.2μ by inhalation of CO_2 for 15 seconds.

Discussion. It has been shown by Cannon, Linton and Linton that liberation of muscle metabolites into the circulation causes an acceleration of the denervated heart. Their work indicated that the acceleration was due to a release of epinephrin from the adrenals. They stimulated the peripheral ends of both sciatic nerves for five minutes.

Our observations confirm their findings. Stimulation of the peripheral end of the cut brachial plexus causes changes in the sartorius muscle like those produced by epinephrin. Two out of three adrenalectomized cats failed to give the epinephrin-like response while the third gave the same results as those with the intact adrenals. In the two animals there was dilatation of the capillaries, and twitching but the twitching was unlike

epinephrin twitching. Moreover, the field became cloudy instead of clearer.

The products of muscular activity cause dilatation of the capillaries in muscle independent of the release of epinephrin, but they cause the muscular tissue to become hazy. The circulation in unstimulated muscle, however, sometimes becomes slower as a result of releasing products of contraction into the circulation. This effect might be central or peripheral or both. The circulation in stimulated muscle, at least with weaker stimuli, seems to always increase. It is impossible to make direct observations on the circulation while maximal contraction is taking place.

Our results support the idea that products of metabolism increase the circulation and affect the muscle fibers throughout the whole musculature of the body. It has already been pointed out by Krogh, Danzer and Hooker and Ebbicke that the capillaries respond to local needs.

SUMMARY

While a muscle is being stimulated through its nerve, the muscle becomes more transparent, the striations become clearer, the capillaries dilate, and the muscle undergoes continuous transverse twitching. If the stimulation is of long duration these effects persist for several minutes after stimulation ceases except that the continuous twitching disappears to be replaced by epinephrin-like twitching from time to time.

Substances released from a contracting muscle produce marked effects upon unstimulated muscle. There are effects apparently due to the release of epinephrin (increased transparency and transverse vibration of the muscle fibers) and effects due to lactic acid or other products of contraction.

Lactic acid causes dilatation of muscle capillaries and a hazy field. Carbon dioxide produces somewhat similar effects.

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